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(21) International Application Number: PCT/US99/22045 (22) International Filing Date: 22 September 1999 (22.09.99) (30) Priority Data: 60/101,858 25 September 1998 (25.09.98) US 09/313,942 19 May 1999 (19.05.99) US (71) Applicant (for all designated States except US): REGENERON PHARMACEUTICALS, INC. [US/US]; 777 Old Saw Mill River Road, Tarrytown, NY 10591-6707 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): STAHL, Neil [US/US]; RD #10, Kent Shore Drive, Carmel, NY 10512 (US). YANCOPOULOS, George, D. [US/US]; 1519 Baptist Church Road, Yorktown Heights, NY 10598 (US). (74) Agents: SORRENTINO, Joseph, M.; Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591 (US) et al.		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: RECEPTOR BASED ANTAGONISTS AND METHODS OF MAKING AND USING (57) Abstract The present invention provides a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex. It also provides a nucleic acid sequence encoding the fusion polypeptide and methods of making and uses for the fusion polypeptide.		

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RECEPTOR BASED ANTAGONISTS AND
METHODS OF MAKING AND USING

5 This application claims priority of U.S. Application No. 09/313,942, filed May 19, 1999, which claims priority of U.S. Provisional Application No. 60/101,858 filed September 25, 1998. Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application.

10

BACKGROUND OF THE INVENTION

Although discovered for varying biological activities, ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), oncostatin M (OSM) and
15 interleukin-6 (IL-6) comprise a defined family of cytokines (referred to herein as the "CNTF family" of cytokines). These cytokines are grouped together because of their distant structural similarities [Bazan, J. Neuron 7: 197-208 (1991); Rose and Bruce, Proc. Natl. Acad. Sci. USA 88: 8641-8645 (1991)], and, perhaps more importantly, because they share " β " signal-
20 transducing receptor components [Baumann, et al., J. Biol. Chem. 265:19853-19862 (1993); Davis, et al., Science 260: 1805-1808 (1993); Gearing et al., Science 255:1434-1437 (1992); Ip et al., Cell 69: 1121-1132 (1992); Stahl, et al., J. Biol. Chem. 268: 7628-7631 (1993); Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. Receptor activation by this family of cytokines results from
25 either homo- or hetero-dimerization of these β components [Davis, et al. Science 260: 1805-1808 (1993), Murakami, et al., Science 260: 1808-1810 (1993); Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. IL-6 receptor activation requires homodimerization of gp130 [Murakami, et al. Science 260: 1808-1810 (1993), Hibi, et al., Cell 63: 1149-1157 (1990)], a protein initially
30 identified as the IL-6 signal transducer [Hibi, et al., Cell 63: 1149-1157 (1990)]. CNTF, LIF and OSM receptor activation results from heterodimerization between gp130 and a second gp130-related protein known as LIFR β [Davis,

et al., Science 260: 1805-1808 (1993)], that was initially identified by its ability to bind LIF [Gearing et al., EMBO J. 10: 2839-2848 (1991)].

In addition to the β components, some of these cytokines also require
5 specificity-determining " α " components that are more limited in their
tissue distribution than the β components, and thus determine the cellular
targets of the particular cytokines [Stahl and Yancopoulos, Cell 74: 587-590
(1993)]. Thus, LIF and OSM are broadly acting factors that may only require
the presence of gp130 and LIFR β on responding cells, while CNTF requires
10 CNTFR α [Stahl and Yancopoulos, Cell 74: 587-590 (1993)] and IL-6 requires
IL-6R α [Kishimoto, et al., Science 258: 593-597 (1992)]. Both CNTFR α
(Davis et al., Science 259:1736-1739 (1993) and IL-6R α [Hibi, et al. Cell
63:1149-1157, Murakami, et al., Science 260:1808-1810 (1990); Taga, et al., Cell
58:573-581 (1989)] can function as soluble proteins, consistent with the
15 notion that they do not interact with intracellular signaling molecules but
that they serve to help their ligands interact with the appropriate signal
transducing β subunits [Stahl and Yancopoulos, Cell 74: 587-590 (1993)].

Additional evidence from other cytokine systems also supports the notion
20 that dimerization provides a common mechanism by which all cytokine
receptors initiate signal transduction. Growth hormone (GH) serves as
perhaps the best example in this regard. Crystallographic studies have
revealed that each GH molecule contains two distinct receptor binding
sites, both of which are recognized by the same binding domain in the
25 receptor, allowing a single molecule of GH to engage two receptor
molecules [de Vos, et al., Science 255: 306-312 (1992)]. Dimerization occurs
sequentially, with site 1 on the GH first binding to one receptor molecule,
followed by the binding of site 2 to a second receptor molecule [Fuh, et al.,
Science 256: 1677-1680 (1992)]. Studies with the erythropoietin (EPO)
30 receptor are also consistent with the importance of dimerization in
receptor activation, as EPO receptors can be constitutively activated by a

single amino acid change that introduces a cysteine residue and results in disulfide-linked homodimers [Watowich, et al., Proc. Natl. Acad. Sci. USA 89:2140-2144 (1992)].

- 5 In addition to homo- or hetero-dimerization of β subunits as the critical step for receptor activation, a second important feature is that formation of the final receptor complex by the CNTF family of cytokines occurs through a mechanism whereby the ligand successively binds to receptor components in an ordered manner [Davis, et al. Science 260:1805-1818 (1993); Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. Thus CNTF first binds to CNTFR α , forming a complex which then binds gp130 to form an intermediate (called here the $\alpha\beta 1$ intermediate) that is not signaling competent because it has only a single β component, before finally recruiting LIFR β to form a heterodimer of β components which then
- 15 initiates signal transduction. Although a similar intermediate containing IL-6 bound to IL-6R α and a single molecule of gp130 has not been directly isolated, we have postulated that it does exist by analogy to its distant relative, CNTF, as well as the fact that the final active IL-6 receptor complex recruits two gp130 monomers. Altogether, these findings led to a
- 20 proposal for the structure of a generic cytokine receptor complex (Figure 1) in which each cytokine can have up to 3 receptor binding sites: a site that binds to an optional α specificity-determining component (α site), a site that binds to the first β signal-transducing component ($\beta 1$ site), and a site that binds to the second β signal-transducing component ($\beta 2$ site) [Stahl
- 25 and Yancopoulos, Cell 74: 587-590 (1993)]. These 3 sites are used in sequential fashion, with the last step in complex formation -- resulting in β component dimerization -- critical for initiating signal transduction [Davis, et al. Science 260:1805-1818 (1993)]. Knowledge of the details of receptor activation and the existence of the non-functional $\beta 1$
- 30 intermediate for CNTF has led to the finding that CNTF is a high affinity

antagonist for IL-6 under certain circumstances, and provides the strategic basis for designing ligand or receptor-based antagonists for the CNTF family of cytokines as detailed below.

- 5 Once cytokine binding induces receptor complex formation, the dimerization of β components activates intracellular tyrosine kinase activity that results in phosphorylation of a wide variety of substrates [Ip, et al. Cell 69:121-1132 (1992)]. This activation of tyrosine kinase appears to be critical for downstream events since inhibitors that block the tyrosine
- 10 phosphorylations also prevent later events such as gene inductions [Ip, et al., Cell 69:121-1132 (1992); Nakajima and Wall, Mol. Cell. Biol. 11:1409-1418 (1991)]. Recently, we have demonstrated that a newly discovered family of non-receptor tyrosine kinases that includes Jak1, Jak2, and Tyk2 (referred to as the Jak/Tyk kinases) [Firmbach-Kraft, et al., Oncogene
- 15 5:1329-1336 (1990); Wilks, et al., Mol. Cell. Biol. 11: 2057-2065 (1991)] and that are involved in signal transduction with other cytokines [Argetsinger, et al., Cell 74:237-244 (1993); Silvennoinen, et al., Proc. Natl. Acad. Sci. USA 90:8429-8433 (1993); Velazquez, et al., Cell 70: 313-322 (1992); Witthuhn, et al., Cell 74:227-236 (1993)], preassociate with the cytoplasmic domains of the
- 20 β subunits gp130 and LIFR β in the absence of ligand, and become tyrosine phosphorylated and activated upon ligand addition [Stahl et al., Science 263:92-95 (1994)]. Therefore these kinases appear to be the most proximal step of intracellular signal transduction activated inside the cell as a result of ligand binding outside of the cell. Assay systems for screening
- 25 collections of small molecules for specific agonist or antagonist activities based on this system are described below.

The CNTF family of cytokines play important roles in a wide variety of physiological processes that provide potential therapeutic applications for

30 both antagonists and agonists.

SUMMARY OF THE INVENTION

An object of the present invention is the production of cytokine antagonists that are useful in the treatment of cytokine-related diseases or disorders.
5

Another object of the invention is the use of the disclosed cytokine antagonists for the treatment of cytokine-related diseases or disorders. For example, an IL-6 antagonist described herein may be used for the treatment
10 of osteoporosis, the primary and second effects of cancers, including multiple myeloma, or cachexia.

Another object of the invention is the development of screening systems useful for identifying novel agonists and antagonists of cytokine receptors.
15

Another object of the invention is the development of screening systems useful for identifying small molecules that act as agonists or antagonists of the cytokines.

20 Another object of the invention is the development of screening systems useful for identifying novel agonists and antagonists of members of the CNTF family of cytokines.

Another object of the invention is the development of screening systems
25 useful for identifying small molecules that act as agonists or antagonists of the CNTF family of cytokines.

BRIEF DESCRIPTION OF THE FIGURES

30 FIGURE 1: Ordered binding of receptor components in a model of a generic cytokine receptor. The model indicates that cytokines contain up to 3 receptor binding sites and interact with their receptor components by

binding first the optional α component, followed by binding to $\beta 1$, and then $\beta 2$. The β components for many cytokine receptors interact through membrane proximal regions (shaded boxes) with the Jak/Tyk family of cytoplasmic protein tyrosine kinases. Only upon dimerization of β components is signal transduction initiated, as schematized by the tyrosine phosphorylations (P) of the β components and the Jak/Tyk kinases.

FIGURE 2: CNTF inhibits IL-6 responses in a PC12 cell line (called PC12D) that expresses IL6R α , gp130, CNTFR α , but not LIFR β . Serum-deprived PC12D cells were incubated + IL-6 (50 ng/mL) in the presence or absence of CNTF as indicated. Some plates also received soluble IL6R α (1 mg/mL) or soluble CNTFR α (1 mg/mL) as indicated. Cell lysates were subjected to immunoprecipitation with anti-gp130 and immunoblotted with anti-phosphotyrosine. Tyrosine phosphorylation of gp130 is indicative of IL-6 induced activation of the IL-6 receptor system, which is blocked upon coaddition of CNTF.

FIGURE 3: Scatchard analysis of iodinated CNTF binding on PC12D cells. PC12D cells were incubated with various concentrations of iodinated CNTF in the presence or absence of excess non-radioactive competitor to determine the specific binding. The figure shows a Scatchard plot of the amount of iodinated CNTF specifically bound, and gives data consistent with two binding sites with dissociation constants of 9 pM and 3.4 nM.

FIGURE 4. The amino acid sequence of human gp130-Fc-His₆. Amino acids 1 to 619 are from human gp130 (Hibi et al., Cell 63:1149-1157 (1990). Note that amino acid number 2 has been changed from a Leu to a Val in order to accommodate a Kozak sequence in the coding DNA sequence. The signal peptide of gp130-Fc-His₆ has been italicized (amino acids 1 to 22). The Ser-Gly bridge is shown in bold type (amino acids 620, 621). Amino acids 662 to 853 are from the Fc domain of human IgG1 (Lewis, et

al., J. Immunol. 151:2829-2838 (1993). (†) mark the two cysteines (amino acids number 632 and 635) of the IgG hinge preceding the Fc that form the inter-chain disulfide bridges that link two Fc domains. The hexahistidine tag is shown in bold/italic type (amino acids 854 to 859). (•) shows the position of the STOP codon.

FIGURE 5: The amino acid sequence of human IL-6R α -Fc. Key: Amino acids 1 to 358 are from human IL-6R α (Yamasaki, et al., Science 241:825-828 (1988). Note that amino acid number 2 has been changed from a Leu to a Val in order to accommodate a Kozak sequence in the coding DNA sequence. The signal peptide of IL-6R α -Fc has been italicized (amino acids 1 to 19). The Ala-Gly bridge is shown in bold type (amino acids 359, 360). Amino acids 361 to 592 are from the Fc domain of human IgG1 (Lewis et al., J. Immunol. 151:2829-2838 (1993). (†) mark the two cysteines (amino acids number 371 and 374) of the IgG hinge preceding the Fc that form the inter-chain disulfide bridges that link two Fc domains. (•) shows the position of the STOP codon.

FIGURE 6: The CNTF/IL-6/IL-11 receptor system. The ordered formation of the hexameric signal transducing receptor complex is depicted schematically. The cytokine associates with the R α component to form an obligatory cytokine•R α complex (Kd is about 5 nM). This low affinity complex next associates with the first signal transducing component, marked β 1, to form a high affinity cytokine•R α • β 1 complex (Kd is about 10 pM). In the case of IL-6R α , this component is gp130. This trimeric high affinity complex subsequently associates with another such complex. Formation of this complex results in signal transduction as it involves dimerization of two signal transducing components, marked β 1 and β 2 respectively (adapted from (Ward et al., J. Bio. Chem. 269:23286-23289 (1994); Stahl and Yancopoulos, J. Neurobiology 25:1454-1466 (1994); Stahl and Yancopoulos, Cell 74:587-590 (1993).

FIGURE 7: Design of heterodimeric receptor-based ligand traps for IL-6. The heterodimeric ligand trap is comprised of two interdisulfide linked proteins, gp130-Fc and IL-6R α -Fc. The gp130-Fc•IL-6R α -Fc complex (upper
5 panel) is shown to mimic the high affinity cytokine•R α • β 1 complex (lower panel). The ligand trap functions as an antagonist by sequestering IL-6 and thus rendering unavailable to interact with the native receptors on IL-6-responsive cells.

10 FIGURE 8. Heteromeric immunoglobulin Heavy/Light Chain Receptor Fusions. An example of a heavy/light chain receptor fusion molecule is schematically depicted. The extracellular domain of gp130 is fused to C γ , whereas the extracellular domain of IL-6R α is fused to the constant region of the kappa chain (κ). The inter-chain disulfide bridges are also depicted
15 (S-S).

FIGURE 9. Amino acid sequence of gp130-C γ 1. Key: Amino acids 1 to 619 are from human gp130 (Hibi, et al., Cell 63:1149-1157 (1990). Ser-Gly bridge is shown in bold type. Amino acids 662 to 651 are from the constant region
20 of human IgG1 (Lewis et al., J. Immunol. 151:2829-2838 (1993). (*) shows the position of the STOP codon.

FIGURE 10: Amino acid sequence of gp130 Δ 3fibro. Key: Amino acids 1 to 330 are from human gp130 (Hibi et al., Cell 63:1149-1157 (1990). Other symbols as described in Figure 9.

25 FIGURE 11: Amino acid sequence of J-CH1. Key: The Ser-Gly bridge is shown in bold, the J-peptide is shown in italics, the CH1 domain is underlined.

FIGURE 12: Amino acid sequence of C γ 4. Key: The Ser-Gly bridge is shown in bold type. Amino acids 2 to 239 comprise the C γ 4 sequence.

FIGURE 13: Amino acid sequence of κ -domain. Key: The Ser-Gly bridge is shown in bold type. Amino acids 2 to 108 comprise the κ domain. The C-terminal cysteine (amino acid 108) is that involved in the disulfide bond of the κ domain with the C γ 1 domain of C γ .

FIGURE 14: Amino acid sequence of λ -domain. Key: The Ser-Gly bridge is shown in bold type. Amino acids 2 to 106 comprise the λ domain (Cheung, et al., J. Virol. 66: 6714-6720 (1992). The C-terminal cysteine (amino acid 106) is that involved in the disulfide bond of the λ domain with the C γ 1 domain of C γ .

FIGURE 15: Amino acid sequence of the soluble IL-6R α domain. Key: Amino acids 1 to 358 comprise the soluble IL-6R α domain (Yamasaki, et al., Science 241:825-828 (1988). The Ala-Gly bridge is shown in bold type.

FIGURE 16: Amino acid sequence of the soluble IL-6R α 313 domain: Key: Amino acids 1 to 313 comprise the truncated IL-6R α domain (IL-6R α 313). The Thr-Gly bridge is shown in bold type.

FIGURE 17: Purification of gp130-C γ 1•IL-6R α - κ . 4% to 12% SDS-PAGE gradient gel run under non-reducing conditions. Proteins were visualized by staining with silver. Lane 1: approximately 100 ng of material purified over Protein A Sepharose (Pharmacia). Lane 2: Molecular size standards (Amersham). Lane 3: The Protein A-purified material shown here after further purification over an IL-6 affinity chromatography step. The positions of the gp130-C γ 1 dimer [(gp130-C γ 1) $_2$], the gp130-C γ 1 dimer

associated with one IL-6R α - κ [(gp130-C γ 1) $_2$ •(IL-6R α - κ) $_1$], and the gp130-C γ 1 dimer associated with two IL-6R α - κ [(gp130-C γ 1) $_2$ •(IL-6R α - κ) $_2$] are shown, as well as the sizes for the molecular size standards in kilodaltons (200, 100, and 46).

5

FIGURE 18: IL-6 dissociates slowly from the ligand trap. The dissociation rate of IL-6 from a heavy/light chain receptor-based ligand trap (gp130-C γ 1•IL-6R α - κ) was compared to that obtained with the neutralizing monoclonal antibody B-E8 (BE8 MAb).

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FIGURE 19: IL-6 can induce multimerization of the ligand trap. (A) Two different ligand traps are depicted schematically and listed according to their ability to bind protein A. gp130-Fc•IL-6R α -Fc (GF6F) binds protein A via its Fc-domains, whereas gp130-CH1•IL-6R α - κ (G16K) does not bind to protein A. (B) Anti-kappa western blotting of proteins precipitated with Protein A-Sepharose from mixtures of GF6F \pm IL-6, G16K \pm IL-6, or GF6F plus G16K \pm IL-6, as marked.

15

FIGURE 20: Inhibition of IL-6-dependent XG-1 cell proliferation. XG-1 cells [Zhang, et al., Blood 83:3654-3663 (1994)] were prepared for a proliferation assay by starving the cells from IL-6 for 5 hours. Assays were set up in 96-well tissue culture dishes in RPMI + 10% fetal calf serum + penicillin/streptomycin + 0.050 nM 2-mercaptoethanol + glutamine. 0.1 ml of that media was used per well. Cells were suspended at a density of 250,000 per ml at the start of the assay. 72 hours post addition of IL-6 \pm ligands traps or antibodies, an MTT assay was performed as described (Panayotatos et al. Biochemistry 33:5813-5818 (1994). The different ligand traps utilized are listed.

20

25

FIGURES 21A-21D: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 424 which is capable of binding the cytokine IL-4 to form a nonfunctional complex.

- 5 FIGURES 22A-22D: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 603 which is capable of binding the cytokine IL-4 to form a nonfunctional complex.

- 10 FIGURES 23A-23D: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 622 which is capable of binding the cytokine IL-4 to form a nonfunctional complex.

- 15 FIGURE 24A-24F: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 412 which is capable of binding the cytokine IL-6 to form a nonfunctional complex.

- 20 FIGURE 25A-25F: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 616 which is capable of binding the cytokine IL-6 to form a nonfunctional complex.

- FIGURE 26A-26E: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 569 which is capable of binding the cytokine IL-1 to form a nonfunctional complex.

- 25 FIGURE 27: Shows that an IL-4 trap designated 4SC375, which is a fusion polypeptide of IL-2R γ -scb-IL4R α -Fc Δ C1, is several orders of magnitude better as an IL-4 antagonist than IL4R α Fc Δ C1 alone in the TF1 cell bioassay.

- 30 FIGURE 28: Shows that an IL-4 trap designated 4SC375 displays antagonistic activity in the TF1 cell bioassay equivalent to an IL-4 trap designated 4SC424 (described in Figs. 21A-21D) which is a fusion

polypeptide of IL-2R γ -IL4R α -Fc Δ C1 having the IL-2R γ component flush with the IL-4R α component.

FIGURE 29: Shows that the IL6 trap (6SC412 IL6R-scb-gpx-Fc Δ C1) described in Figs. 24A-24F is a better antagonist of IL-6 in the XG1 bioassay than the neutralizing monoclonal antibody to human IL-6 - BE8.

FIGURE 30: Shows that the trap 1SC569 (described in Figs. 26A-26E) is able to antagonize the effects of IL-1 and block the IL-6 production from MRC 5 cells upon treatment with IL-1.

FIGURE 31A-31G: The nucleotide and encoded amino acid sequence of the IL-4R α .IL-13R α 1.Fc single chain trap construct is set forth.

FIGURE 32A-32G: The nucleotide and encoded amino acid sequence of the IL-13R α 1.II-4R α .Fc single chain trap construct is set forth.

FIGURE 33: Blocking of IL-13 by IL-4R α .IL-13R α 1.Fc and IL-13R α 1.II-4R α .Fc. Addition of either IL-4R α .IL-13R α 1.Fc or IL-13R α 1.II-4R α .Fc trap at a concentration of 10nM blocks IL-13-induced growth up to ~2nM. At an IL-13 concentration of ~4-5 nM the growth of TF1 cells is inhibited by 50%.

FIGURE 34: Blocking of IL-4 by IL-4R α .IL-13R α 1.Fc and IL-13R α 1.II-4R α .Fc. Addition of either IL-4R α .IL-13R α 1.Fc or IL-13R α 1.II-4R α .Fc at a concentration of 10nM blocks IL-4-induced growth up to ~1nM. At an IL-4 concentration of ~3-4 nM the growth of TF1 cells is inhibited by 50%.

FIGURE 35: Human IL-1 trap blocks the in vivo effects of exogenously administered huIL-1. BALB/c mice were given subcutaneous injection of huIL-1 (0.3 μ g/kg) at time 0. Twenty-four hours prior to huIL-1 injection, the animals were pre-treated with either vehicle or 150-fold molar excess

of huIL-1 trap. Two hours prior to sacrifice (26 hrs), the mice were re-challenged with a second injection of huIL-1 (0.3 µg/kg, s.c.). Blood samples were collected at various time points and sera were assayed for IL-1 levels (expressed as mean +/- SEM; n=5 per group).

5

FIGURE 36A & FIGURE 36B: Human IL-4 trap antagonizes the effects of human IL-4 in monkeys. Figure 36A: Cynomolgus monkeys were treated in three parts as indicated. Human IL-4 (25 µg/kg) was injected subcutaneously twice daily for 4 days and human IL-4 trap (8 mg/ml) and vehicle were given intravenously daily for 5 days, beginning 1 day prior to human IL-4 administration. Plasma was collected daily and assayed for MCP-1 levels. Results were expressed as mean +/- SEM; n=4. (ANOVA p<0.0007; Tukey-Kramer: Part 2 vs. Part 1, p,0.05; Part 2 vs. Part 3, p,0.05; Part 1 vs. Part 3, not significant.) Figure 36B: Cynomolgus monkeys were treated in three parts as indicated. Human IL-4 (25 µg/kg) was injected subcutaneously twice daily for 4 days and human IL-4 trap (8 mg/ml) and vehicle were given intravenously daily for 5 days, beginning 1 day prior to human IL-4 administration. Whole blood was collected daily for flow cytometry analysis for CD16. Results were expressed as mean +/- SEM; n=4. (ANOVA p<0.042; Tukey-Kramer: Part 2 vs. Part 1, p<0.05; Part 2 vs. Part 3 and Part 1 vs. Part 3, not significant.)

FIGURE 37: Murine IL-4 trap partially prevented IL-4-mediated IgE increase in mice. BALB/C mice injected with anti-mouse IgD (100µl/mouse, s.c.) were randomly divided into 3 groups, each received (on days 3-5) either vehicle, murine IL-4 trap (1 mg/kg, s.c.), or a monoclonal antibody to mouse IL-4 (1 mg/kg, s.c.). Sera were collected at various time points and assayed for IgE levels. Results were expressed as mean+/-SEM (n=5 per group). (ANOVA p=0.0002; Tukey-Kramer: vehicle vs. IL-4 trap, p<0.01; vehicle vs. IL-4 antibody, p<0.001; IL-4 trap vs. IL-4 antibody, not significant).

DETAILED DESCRIPTION OF THE INVENTION

- The present invention provides an isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising:
- a) a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the specificity determining component of the cytokine's receptor;
 - b) a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the signal transducing component of the cytokine's receptor; and
 - c) a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a multimerizing component.

By "cytokine binding portion" what is meant is the minimal portion of the extracellular domain necessary to bind the cytokine. It is accepted by those of skill in the art that a defining characteristic of a cytokine receptor is the presence of the two fibronectin-like domains that contain canonical cysteines and of the WSXWS box (Bazan, J.F., 1990, PNAS 87: 6934-6938). Sequences encoding the extracellular domains of the binding component of the cytokine's receptor and of the signal transducing component of the cytokine's receptor may also be used to create the fusion polypeptide of the invention. Similarly, longer sequences encoding larger portions of the components of the cytokine's receptor may be used. However, it is contemplated that fragments smaller than the extracellular domain will function to bind the cytokine and therefore, the invention contemplates fusion polypeptides comprising the minimal portion of the extracellular domain necessary to bind the cytokine as the cytokine binding portion.

The invention comprises a "specificity determining component" of a cytokine's receptor and a "signal transducing component" of the cytokine's receptor. Regardless of the nomenclature used to designate a particular component or subunit of a cytokine receptor, one skilled in the art would
5 recognize which component or subunit of a receptor is responsible for determining the cellular target of the cytokine, and thus would know which component constitutes the "specificity determining component."

Similarly, regardless of the nomenclature used, one of skill in the art
10 would know which component or subunit of a receptor would constitute the "signal transducing component." As used herein, the "signal transducing component" is a component of the native receptor which is not the specificity determining component and which does not bind or weakly binds the cytokine in the absence of the specificity determining
15 component. In the native receptor, the "signal transducing component" may participate in signaling.

For example, while some cytokine receptors have components designated α and β , the IL-4 receptor has a signal transducing component referred to
20 as IL-2R γ . However, regardless of what name is associated with that component, one skilled in the art would know which component of the IL-4 receptor is the signal transducing component. Thus to practice the present invention and create a high affinity trap for IL-4, one of skill in the art would create an isolated nucleic acid comprising a nucleotide sequence
25 encoding a first fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the specificity determining component of the IL-4 receptor (IL-4R α); a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the
30 extracellular domain of the signal transducing component of the IL-4 receptor (IL-2R γ); and a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a

multimerizing component (for example, an Fc domain of IgG) to create a high affinity trap for IL-4.

Some further examples of the receptor components that may be used to
5 prepare cytokine antagonists according to the invention are set forth in
Table 1. The Table 1 sets forth, by way of example but not by way of
limitation, some of the varied nomenclature used in the scientific
literature to describe those components which function as specificity
determining components and those which function as signal transducing
10 components of certain cytokine receptors.

TABLE 1

<u>Cytokine</u>	<u>Specificity determining Component</u>	<u>Signal transducing Component</u>
Interleukin-1 (IL-1)	Type I IL-1R (ref. 8) Type II IL-1R (ref. 8) IL-1RI (ref. 11) IL-1RII (ref. 11)	IL-1R AcP (refs. 8, 11)
Interleukin-2 (IL-2)	α -subunit (ref. 2) α -chain (ref. 3) IL-2R α (ref. 1)	β -chain (ref. 3) β -subunit (ref. 2) γ -chain (ref. 3) IL-2R β (refs. 1, 10) IL-2R γ (refs. 1, 10)
Interleukin-3 (IL-3)	IL-3R α (ref. 1) α -subunit (ref. 2) α -receptor component (ref. 5)	β_c (ref. 1) β -subunit (ref. 2) β -chain (ref. 3) β -receptor component (ref. 5)
Interleukin-4 (IL-4)	IL-4R (ref. 1)	γ -chain (ref. 3) IL-2R γ (ref. 1)
Interleukin-5 (IL-5)	IL-5R α (ref. 1) α -subunit (ref. 2) α -receptor component (ref. 5)	β_c (ref. 1) β -subunit (ref. 2) β -chain (ref. 3) β -receptor component (ref. 5)

TABLE 1 (CONT'D)

<u>Cytokine</u>	<u>Specificity determining Component</u>	<u>Signal transducing Component</u>
Granulocyte macrophage-colony stimulating factor (GM-CSF)	α -receptor component (ref. 5) α -subunit (ref. 2) GMR α (refs. 1, 2)	β -receptor component (ref. 5) β -subunit (ref. 2) β -chain (ref. 3) β_c (ref. 1) GMR β (refs. 1, 2)
Leukemia inhibitory factor (LIF)	LIFBP (ref. 1) α -receptor component (ref. 5)	gp130 (refs. 1, 3) β -receptor component (ref. 5)
Interleukin-11 (IL-11)	α -chain (ref. 4) NR1 (ref. 4)	gp130 (ref. 4)
Interleukin-15 (IL-15)	IL-15R α (ref. 10)	IL-2R β (ref. 10) IL-2R γ (ref. 10)
Interferon- γ (IFN γ)	IFN- γ R (ref. 7) IFN- γ R1 (ref. 7)	AF-1 (ref. 7) IFN- γ R2 (ref. 7)
TGF β	Type II (refs. 6, 9)	Type I (refs. 6, 9)

Only a few of the multitude of references are cited in Table 1, and they are set forth as follows:

1. Sato and Miyajima, Current Opinions in Cell Biology 6: 174-179
5 (1994) - See page 176, lines 9-16;
2. Miyajima, et al., Annual Review of Immunology 10: 295-331 (1992) -
See page 295, line 4 to page 296, line 1; page 305, last paragraph;
3. Kondo, et al, Science 262: 1874-1877 (1993) - See page 1874, cols. 1 & 2;
4. Hilton, et al, EMBO Journal 13: 4765-4775 (1994) - See page 4766, col.
10 1, lines 20-24;
5. Stahl and Yancopoulos, Cell 74: 587-590 (1993) - See page 587,
column 2, lines 15-22;
6. Bassing, et al, Journal of Biological Chemistry 269: 14861-14864 (1994)
- See page 14861, col. 2, lines 1-9 and 21-28;
- 15 7. Kotenko, et al, Journal of Biological Science 270: 20915-20921 (1995) -
See page 20915, lines 1-5 of the abstract;
8. Greenfeder, et al., Journal of Biological Chemistry 270: 13757-13765
(1995) - See page 13757, col. 1, line 6 to col. 2, line 3 and col. 2, lines 10-12;
page 13764, col. 2, last 3 lines and page 13765, col. 1, lines 1-7;
- 20 9. Lebrun and Vale, Molecular Cell Biology 17: 1682-1691 (1997) - See
page 1682, Abstract lines 2-6;
10. Kennedy and Park, Journal of Clinical Immunology 16: 134-143
(1996) - See page 134, lines 1-7 of the abstract; page 136, col 2., lines 1-5;
11. Wesche, et al., Journal of Biological Chemistry 272: 7727-7731 (1997)
25 See page 7731, lines 20-26.

Kotenko, et al. recently identified the IL-10R2 (IL-10R β) chain which is reported to serve as an accessory chain that is essential for the active IL-10 receptor complex and for initiating IL-10 induced signal transduction events (S.V. Kotenko, et al., The EMBO Journal, 1997, Vol. 16: 5894-5903).
30 Additional cytokines and their receptors are described in Appendix II, page A:9 of Immunobiology, The Immune System In Health and Disease, 2nd

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In preparing the nucleic acid sequence encoding the fusion polypeptide of the invention, the first, second, and third components of the fusion polypeptide are encoded in a single strand of nucleotides which, when expressed by a host vector system, produces a monomeric species of the fusion polypeptide. The monomers thus expressed then multimerize due to the interactions between the multimerizing components (the third fusion polypeptide components). Producing the fusion polypeptides in this manner avoids the need for purification of heterodimeric mixtures that would result if the first and second components were produced as separate molecules and then multimerized. For example, U.S. Patent No. 5,470,952 issued November 28, 1995 describes the production of heterodimeric proteins that function as CNTF or IL-6 antagonists. The heterodimers are purified from cell lines cotransfected with the appropriate alpha (α) and beta (β) components. Heterodimers are then separated from homodimers using methods such as passive elution from preparative, nondenaturing polyacrylamide gels or by using high pressure cation exchange chromatography. The need for this purification step is avoided by the methods of the present invention.

In addition, PCT International Application WO 96/11213 published 18 April 1996 entitled Dimeric IL-4 Inhibitors states that the applicant has prepared homodimers in which two IL-4 receptors are bound by a polymeric spacer and has prepared heterodimers in which an IL-4 receptor is linked by a polymeric spacer to an IL-2 receptor gamma chain. The polymeric spacer described is polyethylene glycol (PEG). The two receptor components, IL-4R and IL-2Rgamma are separately expressed and purified. Pegylated homodimers and heterodimers are then produced by joining the components together using bi-functional PEG reagents. It is an advantage

of the present invention that it avoids the need for such time consuming and costly purification and pegylation steps.

In one embodiment of the invention, the nucleotide sequence encoding the first component is upstream of the nucleotide sequence encoding the second component. In another embodiment of the invention, the nucleotide sequence encoding the first component is downstream of the nucleotide sequence encoding the second component. Further embodiments of the invention may be prepared in which the order of the first, second and third fusion polypeptide components are rearranged. For example, if the nucleotide sequence encoding the first component is designated 1, the nucleotide sequence encoding the second component is designated 2, and the nucleotide sequence of the third component is designated 3, then the order of the components in the isolated nucleic acid of the invention as read from 5' to 3' may be any of the following six combinations: 1,2,3; 1,3,2; 2,1,3; 2,3,1; 3,1,2; or 3,2,1.

In further embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the hematopoietin family of cytokines selected from the group consisting of interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-9, interleukin-11, interleukin-13, interleukin-15, granulocyte macrophage colony stimulating factor, oncostatin M, leukemia inhibitory factor, and cardiotrophin-1.

In additional embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the interferon family of cytokines selected from the group consisting of IFN-gamma, IFN-alpha, and IFN-beta.

In additional embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the immunoglobulin superfamily

of cytokines selected from the group consisting of B7.1 (CD80) and B7.2 (B70).

5 In still further embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the TNF family of cytokines selected from the group consisting of TNF-alpha, TNF-beta, LT-beta, CD40 ligand, Fas ligand, CD 27 ligand, CD 30 ligand, and 4-1BBL.

10 In additional embodiments of the invention, the cytokine bound by the fusion polypeptide may be a cytokine selected from the group consisting of interleukin-1, interleukin-10, interleukin-12, interleukin-14, interleukin-18, and MIF.

Because specificity determination and signal transduction occurs by a
15 similar mechanism in the TGF- β /BMP family of cytokines (See D. Kingsley, Genes & Development, 1994, 8: 133-146; J. Wrana, Miner Electrolyte Metab, 24: 120-130 (1998); R. Derynck and X. Feng, Biochimica et Biophysica Acta 1333 (1997) F105-F150; and J. Massague and F. Weis-Garcia, "Serine/threonine Kinase Receptors: Mediators of Transforming Growth
20 Factor Beta Family Signals" In Cancer Surveys, Vol. 27: Cell Signaling, 1996, Imperial Cancer Research Fund) the present invention may be used to produce high affinity antagonists for cytokines that are members of the TGF- β /BMP family.

25 Therefore, in additional embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the TGF- β /BMP family selected from the group consisting of TGF- β 1, TGF- β 2, TGF- β 3, BMP-2, BMP-3a, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8a, BMP-8b, BMP-9, BMP-10, BMP-11, BMP-15, BMP-16, endometrial bleeding
30 associated factor (EBAF), growth differentiation factor-1 (GDF-1), GDF-2, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-12, GDF-14, mullerian

inhibiting substance (MIS), activin-1, activin-2, activin-3, activin-4, and activin-5.

In alternative embodiments of the invention, the specificity determining component, the signal transducing component, or both, may be substituted for by a single chain Fv. A single chain Fv (scFv) is a truncated Fab having only the V region of a heavy chain linked by a stretch of synthetic peptide to a V region of a light chain. See, for example, US Patent Nos. 5,565,332; 5,733,743; 5,837,242; 5,858,657; and 5,871,907 assigned to Cambridge Antibody Technology Limited incorporated by reference herein. Thus the present invention contemplates, for example, an isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the specificity determining component of the cytokine's receptor; a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of an scFv capable of binding the cytokine at a site different from the site at which the cytokine binding portion of the extracellular domain of the specificity determining component of the cytokine's receptor binds; and a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a multimerizing component. Alternatively, the specificity determining component may be substituted for by a scFv that binds to a site on the cytokine different from the site at which the signal transducing component binds. Thus the invention contemplates an isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of a scFv that binds to a site on the cytokine different from the site at which the cytokine binding portion of the extracellular domain of the signal transducing component of the cytokine's receptor binds; a nucleotide sequence encoding a second fusion polypeptide component

comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the signal transducing component of the cytokine's receptor; and a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a
5 multimerizing component.

In another embodiment, the invention contemplates an isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising a nucleotide sequence
10 encoding a first fusion polypeptide component comprising the amino acid sequence of a first scFv that binds to a site on the cytokine; a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence a second scFv that binds to a site on the cytokine different from the site at which the first scFv binds; and a nucleotide
15 sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a multimerizing component.

In all of the above described embodiments comprising scFv's, the invention also contemplates embodiments in which the nucleotide
20 sequence encoding the first component is upstream of the nucleotide sequence encoding the second component; embodiments in which the nucleotide sequence encoding the first component is downstream of the nucleotide sequence encoding the second component; and further embodiments of the invention in which the order of the first, second and
25 third fusion polypeptide components is rearranged. For example, if the nucleotide sequence encoding the first component is designated 1, the nucleotide sequence encoding the second component is designated 2, and the nucleotide sequence of the third component is designated 3, then the order of the components in the isolated nucleic acid of the invention as
30 read from 5' to 3' may be any of the following six combinations: 1,2,3; 1,3,2; 2,1,3; 2,3,1; 3,1,2; or 3,2,1.

In preferred embodiments of the invention, the multimerizing component comprises an immunoglobulin derived domain. More specifically, the immunoglobulin derived domain may be selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG. In another embodiment, the multimerizing component may be an Fc domain from which the first five amino acids (including a cysteine) have been removed to produce a multimerizing component referred to as Fc(Δ C1). Alternatively, the multimerizing component may be an Fc domain in which a cysteine within the first five amino acids has been substituted for by another amino acid such as, for example, serine or alanine.

The present invention also provides for fusion polypeptides encoded by the isolated nucleic acid molecules of the invention. Preferably, the fusion polypeptides are in multimeric form, due to the function of the third multimerizing component. In a preferred embodiment, the multimer is a dimer. Suitable multimerizing components are sequences encoding an immunoglobulin heavy chain hinge region (Takahashi et al., 1982, Cell 29:671-679); immunoglobulin gene sequences, and portions thereof. In a preferred embodiment of the invention, immunoglobulin gene sequences, especially one encoding the Fc domain, are used to encode the third multimerizing component.

The present invention also contemplates a vector which comprises the nucleic acid molecule of the invention as described herein.

Also provided is an expression vector comprising a nucleic acid molecule of the invention as described herein, wherein the nucleic acid molecule is operatively linked to an expression control sequence. Also provided is a host-vector system for the production of a fusion polypeptide which comprises the expression vector of the invention which has been introduced into a host cell suitable for expression of the fusion

polypeptide. The suitable host cell may be a bacterial cell such as E. coli, a yeast cell, such as Pichia pastoris, an insect cell, such as Spodoptera frugiperda, or a mammalian cell, such as a COS, CHO, 293, BHK or NS0 cell.

5

The present invention also provides for methods of producing the fusion polypeptides of the invention by growing cells of the host-vector systems described herein, under conditions permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.

10

The present invention provides novel antagonists which are based on receptor components that are shared by cytokines such as the CNTF family of cytokines.

15 The invention described herein contemplates the production of antagonists to any cytokine that utilizes an α specificity determining component which, when combined with the cytokine, binds to a first β signal transducing component to form a nonfunctional intermediate which then binds to a second β signal transducing component causing β -
20 receptor dimerization and consequent signal transduction. According to the invention, the soluble α specificity determining component of the receptor ($sR\alpha$) and the extracellular domain of the first β signal transducing component of the cytokine receptor ($\beta 1$) are combined to form heterodimers ($sR\alpha:\beta 1$) that act as antagonists to the cytokine by binding the
25 cytokine to form a nonfunctional complex.

As described in Example 1, CNTF and IL-6 share the $\beta 1$ receptor component gp130. The fact that CNTF forms an intermediate with CNTFR α and gp130 can be demonstrated (Example 1) in cells lacking
30 LIFR β , where the complex of CNTF and CNTFR α binds gp130, and

- prevents homodimerization of gp130 by IL-6 and IL-6R α , thereby blocking signal transduction. These studies provide the basis for the development of the IL-6 antagonists described herein, as they show that if, in the presence of a ligand, a nonfunctional intermediate complex, consisting of the ligand, its α receptor component and its β 1 receptor component, can be formed, it will effectively block the action of the ligand. Other cytokines may use other β 1 receptor components, such as LIFR β , which may also be used to produce antagonists according to the present invention.
- Thus for example, in one embodiment of the invention, effective antagonists of IL-6 or CNTF consist of heterodimers of the extracellular domains of the α specificity determining components of their receptors (sIL-6R α and sCNTFR α , respectively) and the extracellular domain of gp130. The resultant heterodimers, which are referred to hereinafter as sIL-6R α : β 1 and sCNTFR α : β 1, respectively, function as high-affinity traps for IL-6 or CNTF, respectively, thus rendering the cytokine inaccessible to form a signal transducing complex with the native membrane-bound forms of their receptors.
- Although soluble ligand binding domains from the extracellular portion of receptors have proven to be somewhat effective as traps for their ligands and thus act as antagonists [Bargetzi, et al., Cancer Res. 53:4010-4013 (1993); , et al., Proc. Natl. Acad. Sci. USA 89: 8616-8620 (1992); Mohler, et al., J. Immunol. 151: 1548-1561 (1993); Narazaki, et al., Blood 82: 1120-1126 (1993)], the IL-6 and CNTF receptors are unusual in that the α receptor components constitute ligand binding domains that, in concert with their ligands, function effectively in soluble form as receptor agonists [Davis, et al. Science 259:1736-1739 (1993); Taga, et al., Cell 58: 573-581 (1989)]. The sR α : β 1 heterodimers prepared according to the present invention provide effective traps for their ligands, binding these ligands with affinities in the picomolar range (based on binding studies for CNTF to PC12D cells)

without creating functional intermediates. The technology described herein may be applied to develop a cytokine trap for any cytokine that utilizes an α -component that confers specificity, as well as a β component which, when bound to the α -specificity component, has a higher affinity for the cytokine than either component alone. Accordingly, antagonists according to the invention include antagonists of interleukins 1 through 5 [IL-1, Greenfeder, et al. J. Biol. Chem. 270:13757-13765 (1995); Guo, et al. J. Biol. Chem. 270:27562-27568 (1995)], IL-2; [Taniguchi, et al. European Patent Nos. 0386289-A and 0386304-A (1990); Takeshita, et al. Science 257:379-382 (1992)]; IL-3; [Kitamura, et al. Cell 66:1165-1174 (1991)], IL-4; [Idzerda, et al. J. Exp. Med. 171:861-873 (1990)], IL-5; [Taverneir, et al. Cell 66:1175-1184 (1991)], IL-11 [(Cherel, et al. Direct Submission to EMBL/GenBank/DDBJ databases; accession No. Z38102)], interleukin 15 [IL-15; Hemar, et al. J. Cell Biol. 129:55-64 (1995); Taniguchi, et al. European Patent Nos. 0386289-A and 0386304-A (1990); Takeshita, et al. Science 257:379-382 (1992)], granulocyte-macrophage colony stimulating factor [GM-CSF; Hayashida, et al. Proc. Natl. Acad. Sci. U.S.A. 97:9655-9659 (1990)], LIF, gamma interferon [IFN γ ; Aguet, et al. Cell 55:273-280 (1988); Soh, et al. Cell 76:793-802 (1994)], and transforming growth factor beta [TGF β ; Inagaki, et al. Proc. Natl. Acad. Sci. USA 90:5359-5363 (1993)].

The α and β receptor extracellular domains may be prepared using methods known to those skilled in the art. The CNTFR α receptor has been cloned, sequenced and expressed [Davis, et al. (1991) Science 253:59-63 which is incorporated by reference in its entirety herein]. The cloning of LIFR β and gp130 are described in Gearing et al. in EMBO J. 10:2839-2848 (1991), Hibi, et al. Cell 63:1149-1157 (1990) and in published PCT application WO 93/10151 published May 27, 1993, all of which are incorporated by reference in their entirety herein.

The receptor molecules useful for practicing the present invention may be prepared by cloning and expression in a prokaryotic or eukaryotic expression system. The recombinant receptor gene may be expressed and purified utilizing any number of methods. The gene encoding the factor
5 may be subcloned into a bacterial expression vector, such as for example, but not by way of limitation, pCP110.

The recombinant factors may be purified by any technique which allows for the subsequent formation of a stable, biologically active protein. For
10 example, and not by way of limitation, the factors may be recovered from cells either as soluble proteins or as inclusion bodies, from which they may be extracted quantitatively by 8M guanidinium hydrochloride and dialysis. In order to further purify the factors, conventional ion exchange chromatography, hydrophobic interaction chromatography, reverse phase
15 chromatography or gel filtration may be used.

The sR α : β heterodimeric receptors may be engineered using known fusion regions, as described in published PCT application WO 93/10151 published May 27, 1993 entitled "Receptor for Oncostatin M and Leukemia Inhibitory
20 Factor" which describes production of β receptor heterodimers, or they may be prepared by crosslinking of extracellular domains by chemical means. The domains utilized may consist of the entire extracellular domain of the α and β components, or they may consist of mutants or fragments thereof that maintain the ability to form a complex with its
25 ligand and other components in the sR α : β 1 complex. For example, as described below in Example 4, IL-6 antagonists have been prepared using gp130 that is lacking its three fibronectin-like domains.

In one embodiment of the invention, the extracellular domains are
30 engineered using leucine zippers. The leucine zipper domains of the human transcription factors c-jun and c-fos have been shown to form stable heterodimers [Busch and Sassone-Corsi, Trends Genetics 6: 36-40

(1990); Gentz, et al., Science 243: 1695-1699 (1989)] with a 1:1 stoichiometry. Although jun-jun homodimers have also been shown to form, they are about 1000-fold less stable than jun-fos heterodimers. Fos-fos homodimers have not been detected.

5

The leucine zipper domain of either c-jun or c-fos are fused in frame at the C-terminus of the soluble or extracellular domains of the above mentioned receptor components by genetically engineering chimeric genes. The fusions may be direct or they may employ a flexible linker domain, such as the hinge region of human IgG, or polypeptide linkers consisting of small amino acids such as glycine, serine, threonine or alanine, at various lengths and combinations. Additionally, the chimeric proteins may be tagged by His-His-His-His-His-His (His₆), [SEQ. ID NO. 1] to allow rapid purification by metal-chelate chromatography, and/or by epitopes to which antibodies are available, to allow for detection on western blots, immunoprecipitation, or activity depletion/blocking in bioassays.

In another embodiment, as described below in Example 3, the sR α : β 1 heterodimer is prepared using a similar method, but using the Fc-domain of human IgG1 [Aruffo, et al., Cell 67:35-44 (1991)]. In contrast to the latter, formation of heterodimers must be biochemically achieved, as chimeric molecules carrying the Fc-domain will be expressed as disulfide-linked homodimers. Thus, homodimers may be reduced under conditions that favor the disruption of inter-chain disulfides but do not effect intra-chain disulfides. Then monomers with different extracellular portions are mixed in equimolar amounts and oxidized to form a mixture of homo- and heterodimers. The components of this mixture are separated by chromatographic techniques. Alternatively, the formation of this type of heterodimers may be biased by genetically engineering and expressing molecules that consist of the soluble or extracellular portion of the receptor components followed by the Fc-domain of hIgG, followed by

- either the c-jun or the c-fos leucine zippers described above [Kostelny, et al., J. Immunol. 148: 1547-1553 (1992)]. Since these leucine zippers form predominately heterodimers, they may be used to drive formation of the heterodimers where desired. As for the chimeric proteins described using
- 5 leucine zippers, these may also be tagged with metal chelates or an epitope. This tagged domain can be used for rapid purification by metal-chelate chromatography, and/or by antibodies, to allow for detection on western blots, immunoprecipitation, or activity depletion/blocking in bioassays.
- 10 In additional embodiments, heterodimers may be prepared using other immunoglobulin derived domains that drive the formation of dimers. Such domains include, for example, the heavy chains of IgG (C γ 1 and C γ 4), as well as the constant regions of kappa (κ) and lambda (λ) light chains of human immunoglobulins. The heterodimerization of C γ with the light
- 15 chain occurs between the CH1 domain of C γ and the constant region of the light chain (CL), and is stabilized by covalent linking of the two domains via a single disulfide bridge. Accordingly, as described in Example 4, constructs may be prepared using these immunoglobulin domains. Alternatively, the immunoglobulin domains include domains that may
- 20 be derived from T cell receptor components which drive dimerization. In another embodiment of the invention, the sR α : β 1 heterodimers are prepared by expression as chimeric molecules utilizing flexible linker loops. A DNA construct encoding the chimeric protein is designed such that it expresses two soluble or extracellular domains fused together in
- 25 tandem ("head to head") by a flexible loop. This loop may be entirely artificial (e.g. polyglycine repeats interrupted by serine or threonine at a certain interval) or "borrowed" from naturally occurring proteins (e.g. the hinge region of hIgG). Molecules may be engineered in which the order of the soluble or extracellular domains fused is switched (e.g.
- 30 sIL6R α /loop/sgp130 or sgp130/loop/sIL-6R α) and/or in which the length

and composition of the loop is varied, to allow for selection of molecules with desired characteristics.

Alternatively, the heterodimers made according to the present invention
5 may be purified from cell lines cotransfected with the appropriate α and β components. Heterodimers may be separated from homodimers using methods available to those skilled in the art. For example, limited quantities of heterodimers may be recovered by passive elution from preparative, nondenaturing polyacrylamide gels. Alternatively,
10 heterodimers may be purified using high pressure cation exchange chromatography. Excellent purification has been obtained using a Mono S cation exchange column.

In addition to sR α : β 1 heterodimers that act as antagonists by binding free
15 CNTF or IL-6, the present invention also contemplates the use of engineered, mutated versions of IL-6 with novel properties that allow it to bind to IL-6R α and a single gp130 molecule, but fail to engage the second gp130 to complete β component homodimerization, and thus act as an effective IL-6 antagonist on any IL-6 responsive cell. Our model for the
20 structure of the IL-6 and CNTF receptor complexes indicates that these cytokines have distinct sites for binding the α , β 1, and β 2 receptor components [Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. Mutations of critical amino acid residues comprising each of these sites gives rise to novel molecules which have the desired antagonistic properties. Ablation
25 of the β 1 site would give a molecule which could still bind to the α receptor component but not the β 1 component, and thereby comprise an antagonist with nanomolar affinity. Mutations of critical amino acid residues comprising the β 2 site of IL-6 (IL-6 β 2-) would give a molecule that would bind to IL-6R α and the first gp130 monomer, but fail to engage the
30 second gp130 and thus be functionally inactive. Similarly, mutations of

the CNTF $\beta 2$ site would give a molecule (CNTF $\beta 2^-$) that would bind CNTFR α and gp130, but fail to engage LIFR β , thereby antagonizing CNTF action by forming the non-functional $\beta 1$ intermediate. Based on the binding results described above where CNTF forms the $\beta 1$ intermediate with high affinity, both CNTF $\beta 2^-$ and IL-6 $\beta 2^-$ would constitute antagonists with affinity in the range of 10 pM.

A variety of means are used to generate and identify mutations of IL-6 or CNTF that have the desired properties. Random mutagenesis by standard methods of the DNA encoding IL-6 or CNTF may be used, followed by analysis of the collection of products to identify mutated cytokines having the desired novel properties as outlined below. Mutagenesis by genetic engineering has been used extensively in order to elucidate the structural organization of functional domains of recombinant proteins. Several different approaches have been described in the literature for carrying out deletion or substitution mutagenesis. The most successful appear to be alanine scanning mutagenesis [Cunningham and Wells (1989), Science 244: 1081-1085] and homolog-scanning mutagenesis [Cunningham, et al., (1989), Science 243:1330-1336].

Targeted mutagenesis of the IL-6 or CNTF nucleic acid sequences using such methods can be used to generate CNTF $\beta 2^-$ or IL-6 $\beta 2^-$ candidates. The choice of regions appropriate for targeted mutagenesis is done systematically, or determined from studies whereby panels of monoclonal antibodies against each factor are used to map regions of the cytokine that might be exposed after binding of the cytokine to the α receptor component alone, or to the $\alpha\beta 1$ heterodimeric soluble receptors described above. Similarly, chemical modification or limited proteolysis of the cytokine alone or in a complex bound to the α receptor component or the $\alpha\beta 1$ heterodimeric soluble receptors described above, followed by analysis

of the protected and exposed regions could reveal potential $\beta 2$ binding sites.

Assays for identifying CNTF or IL-6 mutants with the desired properties
5 involve the ability to block with high affinity the action of IL-6 or CNTF
on appropriately responsive cell lines [Davis, et al., Science 259: 1736-1739
(1993); Murakami, et al., Proc. Natl. Acad. Sci. USA 88: 11349-11353 (1991)].
Such assays include cell proliferation, survival, or DNA synthesis driven
by CNTF or IL-6, or the construction of cell lines where binding of factor
10 induces production of reporters such as CAT or β -galactosidase [Savino, et
al., Proc. Natl. Acad. Sci. USA 90: 4067-4071 (1993)].

Alternatively, the properties of various mutants may be assessed with a
receptor-based assay. One such assay consists of screening mutants for
15 their ability to bind the sR α : $\beta 1$ receptor heterodimers described above
using epitope-tagged [Davis et al., Science 253: 59-63 (1991)] sR α : $\beta 1$ reagents.
Furthermore, one can probe for the presence or absence of the $\beta 2$ site by
assessing whether an epitope-tagged soluble $\beta 2$ reagent will bind to the
cytokine in the presence of the $\beta 1$ heterodimer. For example, CNTF only
20 binds to LIFR β (the $\beta 2$ component) in the presence of both CNTFR α and
gp130 [Davis, et al. Science 260: 1805-1808 (1993); Stahl, et al. J. Biol. Chem.
268: 7628-7631 (1993)]. Thus a soluble LIFR β reagent would only bind to
CNTF in the presence of the soluble sR α : $\beta 1$ dimer sCNTFR α : $\beta 1$. For IL-6,
the sR α : $\beta 1$ reagent would be IL-6R α : $\beta 1$, and the probe for the $\beta 2$ site would
25 be epitope-tagged sgp130. Thus $\beta 2^-$ mutants of CNTF would be identified
as those that bound the sR α : $\beta 1$ reagent, demonstrating that the α and $\beta 1$
site of the cytokine were intact, yet failed to bind the $\beta 2$ reagent.

In addition, the present invention provides for methods of detecting or measuring the activity of potential $\beta 2^-$ mutants by measuring the phosphorylation of a β -receptor component or a signal transduction component selected from the group consisting of Jak1, Jak2 and Tyk2 or
5 any other signal transduction component, such as the CLIPs, that are determined to be phosphorylated in response to a member of the CNTF family of cytokines.

A cell that expresses the signal transduction component(s) described
10 herein may either do so naturally or be genetically engineered to do so. For example, Jak1 and Tyk-2-encoding nucleic acid sequences obtained as described in Velazquez, et al., Cell, Vol. 70:313-322 (1992), may be introduced into a cell by transduction, transfection, microinjection, electroporation, via a transgenic animal, etc., using any known method
15 known in the art.

According to the invention, cells are exposed to a potential antagonist and the tyrosine phosphorylation of either the β -component(s) or the signal transduction component(s) are compared to the tyrosine phosphorylation
20 of the same component(s) in the absence of the potential antagonist. In another embodiment of the invention, the tyrosine phosphorylation that results from contacting the above cells with the potential antagonist is compared to the tyrosine phosphorylation of the same cells exposed to the parental CNTF family member. In such assays, the cell must either express
25 the extracellular receptor (α -component) or the cells may be exposed to the test agent in the presence of the soluble receptor component. Thus, for example, in an assay system designed to identify agonists or antagonists of CNTF, the cell may express the α - component CNTFR α , the β - components gp130 and LIFR β and a signal transducing component such as
30 Jak1. The cell is exposed to test agents, and the tyrosine phosphorylation of either the β - components or the signal transducing component is

compared to the phosphorylation pattern produced in the presence of CNTF. Alternatively, the tyrosine phosphorylation which results from exposure to a test agent is compared to the phosphorylation which occurs in the absence of the test agent. Alternatively, an assay system, for
5 example, for IL-6 may involve exposing a cell that expresses the β -component gp130 and a signal transducing protein such as Jak1, Jak2 or Tyk2 to a test agent in conjunction with the soluble IL-6 receptor.

In another embodiment of the invention the above approaches are used to
10 develop a method for screening for small molecule antagonists that act at various steps in the process of ligand binding, receptor complex formation, and subsequent signal transduction. Molecules that potentially interfere with ligand-receptor interactions are screened by assessing interference of complex formation between the soluble receptors and ligand as described
15 above. Alternatively, cell-based assays in which IL-6 or CNTF induce response of a reporter gene are screened against libraries of small molecules or natural products to identify potential antagonists. Those molecules showing antagonist activity are rescreened on cell-based assays responding to other factors (such as GM-CSF or factors like Neurotrophin-
20 3 that activate receptor tyrosine kinases) to evaluate their specificity against the CNTF/IL-6/OSM/LIF family of factors. Such cell-based screens are used to identify antagonists that inhibit any of numerous targets in the signal transduction process.

25 In one such assay system, the specific target for antagonists is the interaction of the Jak/Tyk family of kinases [Firmbach-Kraft, Oncogene 5: 1329-1336 (1990); Wilks, et al., Mol. Cell. Biol. 11:2057-2065 (1991)] with the receptor β subunits. As described above, LIFR β and gp130 preassociate with members of the Jak/Tyk family of cytoplasmic protein tyrosine
30 kinases, which become activated in response to ligand-induced β component dimerization Stahl, et al. Science 263:92-95 (1993). Thus small molecules that could enter the cell cytoplasm and disrupt the interaction

between the β component and the Jak/Tyk kinase could potentially block all subsequent intracellular signaling. Such activity could be screened with an *in vitro* scheme that assessed the ability of small molecules to block the interaction between the relevant binding domains of purified β component and Jak/Tyk kinase. Alternatively, one could easily screen for molecules that could inhibit a yeast-based assay of β component binding to Jak/Tyk kinases using the two-hybrid interaction system [Chien, et al., Proc. Natl. Acad. Sci. 88: 9578-9582 (1991)]. In such a system, the interaction between two proteins (β component and Jak/Tyk kinase or relevant domains thereof in this example) induces production of a convenient marker such as β -galactosidase. Collections of small molecules are tested for their ability to disrupt the desired interaction without inhibiting the interaction between two control proteins. The advantage of this screen would be the requirement that the test compounds enter the cell before inhibiting the interaction between the β component and the Jak/Tyk kinase.

The CNTF family antagonists described herein either bind to, or compete with the cytokines CNTF and IL-6. Accordingly, they are useful for treating diseases or disorders mediated by CNTF or IL-6. For example, therapeutic uses of IL-6 antagonists would include the following:

- 1) In osteoporosis, which can be exacerbated by lowering of estrogen levels in post-menopausal women or through ovariectomy, IL-6 appears to be a critical mediator of osteoclastogenesis, leading to bone resorption [Horowitz, Science 260: 626-627 (1993); Jilka, et al., Science 257: 88-91 (1992)]. Importantly, IL-6 only appears to play a major role in the estrogen-depleted state, and apparently is minimally involved in normal bone maintenance. Consistent with this, experimental evidence indicates that function-blocking antibodies to IL-6 can reduce the number of osteoclasts [Jilka, et al. Science 257: 88-91 (1992)]. While estrogen replacement therapy is also used, there appear to be side effects that may include increased risk of

endometrial and breast cancer. Thus, IL-6 antagonists as described herein would be more specific to reduce osteoclastogenesis to normal levels.

2) IL-6 appears to be directly involved in multiple myeloma by acting in either an autocrine or paracrine fashion to promote tumor formation [van Oers, et al., *Ann Hematol.* 66: 219-223 (1993)].
Furthermore, the elevated IL-6 levels create undesirable secondary effects such as bone resorption, hypercalcemia, and cachexia; in limited studies function-blocking antibodies to IL-6 or IL-6Ra have some efficacy [Klein, et al., *Blood* 78: 1198-1204 (1991); Suzuki, et al., *Eur. J. Immunol.* 22:1989-1993 (1992)]. Therefore, IL-6 antagonists as described herein would be beneficial for both the secondary effects as well as for inhibiting tumor growth.

3) IL-6 may be a mediator of tumor necrosis factor (TNF) that leads to cachexia associated with AIDS and cancer [Strassmann, et al., *J. Clin. Invest.* 89: 1681-1684 (1992)], perhaps by reducing lipoprotein lipase activity in adipose tissue [Greenberg, et al., *Cancer Research* 52: 4113-4116 (1992)]. Accordingly, antagonists described herein would be useful in alleviating or reducing cachexia in such patients.

Effective doses useful for treating these or other CNTF family related diseases or disorders may be determined using methods known to one skilled in the art [see, for example, Fingl, et al., *The Pharmacological Basis of Therapeutics*, Goodman and Gilman, eds. Macmillan Publishing Co., New York, pp. 1-46 ((1975)]. Pharmaceutical compositions for use according to the invention include the antagonists described above in a pharmacologically acceptable liquid, solid or semi-solid carrier, linked to a carrier or targeting molecule (e.g., antibody, hormone, growth factor, etc.) and/or incorporated into liposomes, microcapsules, and controlled release preparation (including antagonist expressing cells) prior to administration *in vivo*. For example, the pharmaceutical composition may comprise one or more of the antagonists in an aqueous solution, such as sterile water, saline, phosphate buffer or dextrose solution. Alternatively, the active agents may be comprised in a solid (e.g. wax) or semi-solid (e.g. gelatinous) formulation that may be implanted into a patient in need of such

treatment. The administration route may be any mode of administration known in the art, including but not limited to intravenously, intrathecally, subcutaneously, by injection into involved tissue, intraarterially, intranasally, orally, or via an implanted device.

5

Administration may result in the distribution of the active agent of the invention throughout the body or in a localized area. For example, in some conditions which involve distant regions of the nervous system, intravenous or intrathecal administration of agent may be desirable. In some situations, an implant containing active agent may be placed in or near the lesioned area. Suitable implants include, but are not limited to, gelfoam, wax, or microparticle-based implants.

10

EXAMPLES

15

EXAMPLE 1: CNTF COMPETES WITH IL-6 FOR BINDING TO GP130

MATERIALS AND METHODS

Materials. A clone of PC12 cells that respond to IL-6 (PC12D) was obtained from DNAX. Rat CNTF was prepared as described [Masiakowski, et al., J. Neurochem. 57:1003-10012 (1991)]. IL-6 and sIL-6R α were purchased from R & D Systems. Antisera was raised in rabbits against a peptide derived from a region near the C-terminus of gp130 (sequence: CGTEGQVERFETVGME) [SEQ. ID. NO. 2] by the method described (Stahl, et al. J. Biol. Chem. 268:7628-7631 (1993). Anti-phosphotyrosine monoclonal 4G10 was purchased from UBI, and reagents for ECL from Amersham.

25

Signal Transduction Assays. Plates (10 cm) of PC12D were starved in serum-free medium (RPMI 1640 + glutamine) for 1 hour, then incubated with IL-6 (50 ng/mL) + sIL-6R (1 mg/mL) in the presence or absence of

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added rat CNTF at the indicated concentrations for 5 minutes at 37°C. Samples were then subjected to anti-gp130 immunoprecipitation, SDS PAGE, and anti-phosphotyrosine immunoblotting as described (Stahl, et al. J. Biol. Chem. 268:7628-7631 (1993)).

5

RESULTS

The ability of CNTF to block IL-6 responses was measured using a PC12 cell line (called PC12D) that expresses IL-6R α , gp130, and CNTFR α , but not LIFR β . As one would predict, these cells respond to IL-6, but not to CNTF (Fig. 2) since LIFR β is a required component for CNTF signal transduction [Davis, et al., Science 260: 59-63 (1993)]. In accordance with results on other cell lines [Ip, et al., Cell 69: 1121-1132 (1992)], PC12D cells give tyrosine phosphorylation of gp130 (as well as a variety of other proteins called CLIPs) in response to 2 nM IL-6 (Fig. 2). Addition of recombinant soluble IL-6R α (sIL-6R α) enhances the level of gp130 tyrosine phosphorylation, as has been reported in some other systems [(Taga, et al., Cell 58: 573-581 (1989))]. However, addition of 2 nM CNTF simultaneously with IL-6 severely diminishes the tyrosine phosphorylation of gp130. Although a slight gp130 phosphorylation response remains in the presence of CNTF, IL-6, and sIL-6R α , it is eliminated if the CNTF concentration is increased fourfold to 8 nM. Thus, in IL-6 responsive cells that contain CNTFR α but no LIFR β , CNTF is a rather potent antagonist of IL-6 action.

25 EXAMPLE 2. BINDING OF CNTF TO THE CNTFR α : β

MATERIALS AND METHODS

Scatchard Analysis of CNTF Binding. 125I-CNTF was prepared and purified as described [Stahl et al. JBC 268: 7628-7631 (1993)]. Saturation binding studies were carried out in PC12 cells, using concentrations of 125I-

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CNTF ranging from 20pM to 10nM. Binding was performed directly on a monolayer of cells. Medium was removed from wells and cells were washed once with assay buffer consisting of phosphate buffered saline (PBS; pH 7.4), 0.1mM bacitracin, 1mM PMSF, 1mg/ml leupeptin, and 1mg/ml BSA. Cells were incubated in ^{125}I -CNTF for 2 hours at room temperature, followed by 2 quick washes with assay buffer. Cells were lysed with PBS containing 1% SDS and counted in a Packard Gamma Counter at 90-95% efficiency. Non-specific binding was defined by the presence of 100-fold excess of unlabelled CNTF. Specific binding ranged from 70% to 95%.

RESULTS

The equilibrium constant for binding of CNTF to CNTFR α : β 1 was estimated from Scatchard analysis of iodinated CNTF binding on PC12D cells (Figure 3). The data is consistent with a 2 site fit having dissociation constants of 9 pM and 3.4 nM. The low affinity site corresponds to interaction of CNTF with CNTFR α , which has a K_d near 3 nM [(Panayotatos, et al., J. Biol. Chem. 268: 19000-19003 (1993)]. We interpret the high affinity complex as the intermediate containing CNTF, CNTFR α , and gp130. A Ewing sarcoma cell line (EW-1) which does contain CNTFR α , gp130, and LIFR β , and therefore gives robust tyrosine phosphorylation in response to CNTF, displays a very similar two site fit with dissociation constants of 1 nM and 10. Thus it is apparent that CNTF binds with equally high affinity to a complex containing only CNTFR α and gp130, as it does to a complex which additionally contains LIFR β , thus demonstrating the feasibility of creating the sR α : β antagonists described herein.

EXAMPLE 3. METHODS OF PRODUCING CYTOKINE LIGAND TRAPS

Virus Stock Production

- 5 SF21 insect cells obtained from *Spodoptera frugiperda* were grown at 27°C in Gibco SF900 II medium to a density of 1×10^6 cells/mL. The individual virus stock for either GP130-Fc-His₆ (Figure 4) or IL6Ra-Fc (Figure 5) was added to the bioreactor to a low multiplicity 0.01-0.1 PFU/cell to begin the infection. The infection process was allowed to continue for 5-7 days
- 10 allowing maximum virus replication without incurring substantial cell lysis. The cell suspension was aseptically aliquoted into sterile centrifuge bottles and the cells removed by centrifugation. The cell-free supernatant was collected in sterile bottles and stored at 4°C until further use.
- 15 The virus titer was determined by plaque assay as described by O'Reilly, Miller and Luckow. The method is carried out in 60mm tissue-culture dishes which are seeded with 2×10^6 cells. Serial dilutions of the virus stock are added to the attached cells and the mixture incubated with rocking to allow the virus to adsorb to individual cells. An agar overlay is
- 20 added and plates incubated for 5 - 7 days at 27°C. Staining of viable cells with neutral red revealed circular plaques resulting which were counted to give the virus titer.

Coinfection of Cells for Protein Production

- 25 Uninfected SF21 Cells were grown in a 60L ABEC bioreactor containing 40L of SF900 II medium. Temperature was controlled at 27°C and the dissolved oxygen level was maintained at 50% of saturation by controlling the flowrate of oxygen in the inlet gas stream. When a density of 2×10^6
- 30 cells/mL was reached, the cells were concentrated within the bioreactor to a volume of 20L using a low shear steam sterilizable pump with a tangential flow filtration device with Millipore Prostak 0.65 micron

membranes. After concentration fresh sterile growth medium is slowly added to the bioreactor while the filtration system continues to remove the spent growth medium by diafiltration. After two volume exchanges (40L) have been carried out an additional 20L of fresh medium was added
5 to the bioreactor to resuspend the cells to the original volume of 40L. The cell density was determined once again by counting viable cells using a hemacytometer.

The required amount of each virus stock was calculated based on the cell
10 density, virus titer and the desired multiplicity of infection (MOI). Virus stock ratios of 5:1, 5:2, 10:2 and 10:4, IL6R α -Fc to GP130-Fc-His₆ all resulted in production of significant amounts of heterodimer. The ideal virus stock ratio is highly dependent on the ease of purification of the heterodimer from each of the two homodimers. The IL6R α -Fc
15 homodimer is relatively easy to remove downstream by immobilized metal affinity chromatography. Virus infection ratios have been chosen to minimize the formation of the GP130-Fc-His₆ homodimer which is more difficult to clear downstream. The relative amount of GP130-Fc-His₆ virus stock chosen for infection has increased with successive batches as the
20 purification method for clearing the resultant homodimer has improved.

The virus stocks were aseptically mixed in a single vessel then transferred to the bioreactor. This results in synchronous infection of the SF21 cells. The infection is allowed to proceed for three to four days, allowing
25 sufficient time for maximal production of the heterodimer protein.

Recovery and Protein A Chromatographic Purification

At the conclusion of the infection phase of the bioreactor process the cells
30 were concentrated in the bioreactor using a 10 ft² Millipore Prostack filter (0.65 micron) pore size. The cell-free permeate passing through the filter was collected in a clean process vessel. At the conclusion of the filtration

operation the pH of permeate stream, containing the protein product, was adjusted to 8.0 with 10N NaOH. The resultant precipitate was removed by forcing the extract through a 0.8 micron depth filter (Sartorius), followed by a 0.2 micron filter. Sufficient 0.5M EDTA stock was added to give a final
5 concentration of 5mM. The filtered protein solution was loaded onto a 10 cm diameter column containing 100-200 mL of Pharmacia Protein A Sepharose 4 Fast Flow, equilibrated with PBS. Protein A has a very high affinity for the Fc-Fc domain of each of the 3 recombinant protein products, allowing them to bind while other proteins in the cell-free
10 extract flow through the column. After loading the column was washed to baseline with PBS containing an additional 350mM NaCl. The IgG-Fc tagged proteins were eluted at low pH, either with 0.5M acetic acid or with a decreasing pH gradient of 0.1M citric acid and 0.2M disodium phosphate buffers. Tris base or disodium phosphate was added to the eluted protein
15 to avoid prolonged exposure to low pH conditions.

The pooled protein was diafiltered into PBS or HEPES buffer and derivitized with 1 mM iodoacetamide to protect the exposed sulfhydryl group on the free cysteine near the hinge region of each Fc domain. This
20 prevents disulfide mediated aggregation of proteins. A 6 ft² Millipore spiral wound ultrafiltration membrane with nominal 30 kiloDalton cutoff was used to perform the buffer exchange. The total protein was determined by UV absorbance at 280 nm using the diafiltration buffer as a blank. The relative amounts of heterodimer and two homodimer
25 proteins were determined by SDS PAGE gel electrophoresis using a 6% Tris-Glycine gel (Novex). Gels were Coomassie-stained then transferred into destain solution overnight. A Shimadzu scanning densitometer was used to determine the relative intensity of the individual protein bands on the SDS PAGE gel. The peak area ratios are used to compute the fraction of
30 heterodimer and each of the homodimers in the column pool fractions.

Immobilized Metal Affinity Chromatographic Purification

The six histidine residues on the C-terminus of the GP130-Fc-His₆ fusion protein provides an excellent molecular handle for separation of the heterodimeric IL6 antagonist from the two homodimers. The imidazole group on each of the C-terminal histidines of the GP130-Fc-His₆ moiety has a strong binding constant with several divalent metals, including copper, nickel, zinc, cobalt, iron and calcium. Since the IL6R α -Fc homodimer has no C-terminal histidine residues, it clearly has the lowest affinity. The IL6R α -Fc-GP130-Fc-His₆ heterodimer has a single stand set six histidines giving it greater affinity for the metal, while the GP130-Fc-His₆ homodimer has two sets of six histidines each giving it the highest affinity of the three IgG tagged proteins to the metal affinity column. Selective elution of the three proteins with increasing amounts of imidazole in the elution buffer therefore elutes the proteins in the following order:

1. IL6R α -Fc homodimer
2. IL6R α -Fc-GP130-Fc-His heterodimer
3. GP130-Fc-His homodimer

A 26 mm diameter column containing 100 mL of Pharmacia Chelating Sepharose Fast Flow was saturated with a solution of nickel sulfate until a significant green color is observed in the column eluate. The column is then washed with several column volumes of deionized water, then equilibrated with 50 mM HEPES, 40mM imidazole, pH 8.0. The binding of imidazole to the immobilized nickel results in a green to blue color change. Imidazole was added to the protein load to a final concentration of 40mM. Addition of imidazole to the protein load reduces the binding of IL6R α -Fc homodimer, increasing the surface area available for the remaining two species. After loading, the column was washed with

several column volumes of 50 mM HEPES, 80mM imidazole, pH 8.0 until a steady baseline was reestablished. The heterodimer was selectively eluted with 50 mM HEPES, 150mM imidazole, pH 8.0 over several column volumes. The protein fractions were pooled and diafiltered into PBS as
5 described in the section above.

EXAMPLE 4. ALTERNATIVE METHODS OF CONSTRUCTING LIGAND TRAPS

10 As described above, receptor activation by CNTF, and analogously by IL-6 and IL-11, follows an ordered sequence of binding events (Figure 6). The cytokine initially binds to its cognate $R\alpha$ with low affinity ($K_d = 3$ to 10 nM); this is a required step - cells which do not express the cognate $R\alpha$ do not respond to the cognate cytokine. The cytokine• $R\alpha$ complex associates
15 with the first signal transducing component, gp130, to form a high affinity complex (K_d in the order of 10 pM for the CNTF•CNTF $R\alpha$ •gp130 complex). This complex does not transduce signal, as it is the dimerization of the signal transducing components that brings about signaling (Stahl and Yancopoulos, J. Neurobiology 25: 1454-1466 (1994); Stahl et al., Science
20 267:1349-1353 (1995); Davis et al., Science 260:1805-1808 (1993); Stahl et al., Science 263:92-95 (1994); Murakami, et al. Science 260:1808-1810 (1993). At least in the case of IL-6, the cytokine• $R\alpha$ •signal transducer heterotrimeric complex subsequently associates with another like complex, to form a hexameric complex (Figure 6) (Ward et al., J. Biol. Chem. 269:23286-23289
25 (1994). The resulting dimerization of the signal transducers - gp130 in the case of IL-6 (Murakami et al., Science 260:1808-1810 (1993) and IL-11, gp130 and LIFR in the case of CNTF (Davis et al., Science 260:1805-1808 (1993) - brings about signal transduction.

30 The initial heterodimeric molecules made comprised a soluble $R\alpha$ -component linked to the extracellular domain of gp130. These molecules

were shown to mimic the high affinity cytokine•R α •gp130 complex and behave as a high affinity antagonist of their cognate cytokine (Figure 7). To make these molecules, the extracellular domain of gp130 was paired with the extracellular domain of the α -receptor components for IL-6 and CNTF, 5 IL-6R α and CNTFR α respectively. To link the R α with the extracellular domain of gp130, the soluble R α -components and gp130 were fused to the Fc portion of human IgG1 to produce R α -Fc and gp130-Fc respectively. The Fc domain was chosen primarily but not solely because it naturally forms disulfide-linked dimers. Heterodimeric molecules comprising R α - 10 Fc•gp130-Fc were expressed, purified and shown to behave as highly potent antagonists of their cognate ligand. Furthermore, these molecules were found to be highly specific for their cognate cytokine since it is the choice of the α -receptor component which specifies which cytokine is bound and trapped (there is no measurable binding of the cytokine to 15 gp130 in the absence of the appropriate R α).

Here we describe an extension of this technology which allows the engineering of different heteromeric soluble receptor ligand traps which by virtue of their design may have additional beneficial characteristics such as 20 stability, Fc-receptor-mediated clearance, or reduced effector functions (such as complement fixation). Furthermore, the technology described should prove suitable for the engineering of any heteromeric protein in mammalian or other suitable protein expression systems, including but not limited to heteromeric molecules which employ receptors, ligands, 25 and catalytic components such as enzymes or catalytic antibodies.

MATERIALS AND METHODS

Genetic engineering of heteromeric immunoglobulin heavy/light chain 30 soluble receptor-based ligand traps for IL-6.

The IL-6 traps described here were engineered using human gp130, human IL-6 α -receptor (IL-6R α), the constant region of the heavy chains (C γ) of human IgG1 (C γ 1) (Lewis et al., Journal of Immunology 151:2829-2838 (1993) or IgG4 (C γ 4) with or without a join-region (J), and the constant regions of kappa (κ) and lambda (λ) (Cheung, et al., Journal of Virology 66:6714-6720 (1992) light chains of human immunoglobulin (Ig), also with or without a different j-peptide (j)). This design takes advantage of the natural ability of the C γ domain to heterodimerize with κ or λ light chains. The heterodimerization of C γ with the light chain occurs between the CH1 domain of C γ and the constant region of the light chain (C L), and is stabilized by covalent linking of the two domains via a single disulfide bridge. We reasoned that, like the Fc domain of human IgG1, the combination of C γ with C L could be used to produce disulfide linked heteromeric proteins comprised of the extracellular domain of gp130 on one chain and the extracellular domain of IL-6R α on the other chain. Like their Fc-based counterparts, such proteins were postulated to be high affinity ligand traps for IL-6 and as a result to inhibit the interaction of IL-6 with the native receptor on IL-6-responsive cells, thus functioning as IL-6 antagonists. Furthermore, constructs employing the full length C γ region would, much like antibodies, form homodimers of the C γ chain, giving rise to antibody-like molecules comprising of two "light chains" and two "heavy chains" (Figure 8). The potential advantage of this design is that it may more closely mimic the IL-6•IL-6R α •gp130 complex and may display a higher affinity for the ligand than comparable single heterodimers. An additional design is incorporated by using truncated versions of C γ , comprised only of the CH1 domain. These will form heterodimeric molecules with receptor- κ fusion proteins, and will thus resemble the Fab fragment of antibodies.

All the soluble receptor-Ig chimeric genes may be engineered in plasmid vectors including, but not limited to, vectors suitable for mammalian expression (COS monkey kidney cells, Chinese Hamster Ovary cells [CHO], and ras-transformed fibroblasts [MG-ras]) and include a Kozak sequence (CGC CGC CAC CAT GGT G) at the beginning of each chimeric gene for efficient translation. Engineering was performed using standard genetic engineering methodology. Each construct was verified by DNA sequencing, mammalian expression followed by western blotting with suitable antibodies, biophysical assays that determine ligand binding and dissociation, and by growth inhibition assays (XG-1, as described later). Since the domains utilized to engineer these chimeric proteins are flanked by appropriate restriction sites, it is possible to use these domains to engineer other chimeric proteins, including chimeras employing the extracellular domains of the receptors for factors such as IL-1, IL-2, IL-3, IL-4, IL-5, GM-CSF, LIF, IL-11, IL-15, IFN γ , TGF β , and others. The amino acid coordinates for each component utilized in making the IL-6 traps are listed below (Note: numbering starts with the initiating methionine as #1; long sequences are listed using the single letter code for the twenty amino acids):

20

(a) Constructs employing human gp130:

- (i) **gp130-C γ 1** was engineered by fusing in frame the extracellular domain of gp130 (amino acids 1 to 619) to a Ser-Gly bridge, followed by the 330 amino acids which comprise C γ 1 and a termination codon (Figure 9).
- 25 (ii) **gp130-J-C γ 1** was engineered in the same manner as gp130-C γ 1 except that a J-peptide (amino acid sequence: GQGTLVTVSS) was inserted between the Ser-Gly bridge and the sequence of C γ 1 (see Figure 9).
- (iii) **gp130 Δ 3fibro-C γ 1** was engineered by fusing in frame the extracellular domain of gp130 without its three fibronectin-like domains (Figure 10).
- 30 The remaining part of this chimeric protein is identical to gp130-C γ 1.

- (iv) **gp130-J-CH1** was engineered in a manner identical for that described for gp130-C γ 1, except that in place of the C γ 1 region only the CH1 part of C γ 1 has been used (Figure 11). The C-terminal domain of this construct includes the part of the hinge that contains the cysteine residue responsible for heterodimerization of the heavy chain of IgG with a light chain. The part of the hinge that contains the two cysteines involved in C γ 1 homodimerization has been deleted along with the CH2 and CH3 domains.
- (v) **gp130-C γ 4** was engineered in a manner identical to that described for gp130-C γ 1, except that C γ 4 was used in place of C γ 1 (Figure 12). In addition, an *Rsr*II DNA restriction site was engineered at the hinge region of the C γ 4 domain by introducing two silent base mutations. The *Rsr*sII site allows for other desired genetic engineering manipulations, such as the construction of the CH1 equivalent of gp130-C γ 4.
- (vi) **gp130- κ** was engineered in a manner identical to that described for gp130-C γ 1, except that the constant region of the κ light chain of human Ig was used in place of C γ 1 (Figure 13).
- (vi) **gp130-J- κ** was engineered in a manner identical to that described for gp130-J- κ , except that a j-peptide (amino acid sequence: TFGQG $\overline{\text{TK}}$ VEIK) was inserted between the Ser-Gly bridge and the κ -region.
- (viii) **gp130- λ** was engineered in a manner identical to that described for gp130-C γ 1, except that the constant region of the λ light chain (Cheung, et al., Journal of Virology 66:6714-6720 (1992) of human Ig was used in place of C γ 1 (Figure 14).

25

(b) Constructs employing human IL-6R α :

- (i) **IL6R α -C γ 1** was engineered by fusing in frame amino acids 1 to 358 of IL-6R α (Yamasaki et al., Science 241:825-828 (1988), which comprise the

extracellular domain of IL-6R α (Figure 15), to an Ala-Gly bridge, followed by the 330 amino acids which comprise C γ 1 and a termination codon.

(ii) IL6R α - κ was engineered as described for IL6R α -C γ 1, except that the κ -domain (Figure 13) utilized for gp130- κ was used in place of C γ 1.

5 (iii) IL6R α -j- κ was engineered as described for IL6R α - κ except that the j-peptide described for gp130-j- κ was placed between the Ala-Gly bridge and the κ -domain.

(iv) Three additional constructs, IL6R α 313-C γ 1, IL6R α 313- κ , and IL6R α 313-j- κ , were engineered as using a truncated form of IL-6R α comprised of
10 amino acids 1 to 313 (Figure 16). Each of these constructs were made by fusing in frame IL6R α 313 with a Thr-Gly bridge followed by the C γ 1, κ -, and j- κ -domains described above. These constructs were engineered in order to complement the gp130 Δ 3fibro-derived constructs.

15 Expression and purification of ligand traps

To produce covalently linked heterodimers of soluble gp130 and soluble IL-6R α , gp130-Ig chimeric proteins were co-expressed with appropriate IL-6R α -Ig chimeric proteins in complementing pairs. Co-expression was
20 achieved by co-transfecting the corresponding expression vectors into suitable mammalian cell lines, either stably or transiently. The resulting disulfide-linked heterodimers were purified from conditioned media by several different methods, including but not limited to affinity chromatography on immobilized Protein A or Protein G, ligand-based
25 affinity chromatography, ion exchange, and gel filtration.

An example of the type of methods used for purification of a heavy/light receptor fusion protein is as follows: gp130-C γ 1•IL-6R α - κ was expressed in COS cells by co-transfecting two different vectors, encoding gp130-C γ 1 and

IL-6R α - κ respectively. Serum-free conditioned media (400 ml) were collected two days post-transfection and C γ 1-bearing proteins were purified by affinity chromatography over a 1ml Protein A Sepharose (Pharmacia). The material generated in this step was further purified by a second
5 affinity chromatography step over a 1 ml NHS-activated Sepharose (Pharmacia) which was derivatized with recombinant human IL-6, in order to remove gp130-C γ 1 dimer from gp130-C γ 1•IL-6R α - κ complexes (the gp130-C γ 1 dimer does not bind IL-6). Proteins generated by this method were more than 90% pure, as evidenced by SDS-PAGE followed by silver-
10 staining (Figure 17). Similar protocols have been employed successfully towards the purification of other heavy/light receptor heterodimers.

RESULTS

15 Biological activity of immunoglobulin heavy/light chain receptor fusion antagonists

The purified ligand traps were tested for their ability to bind IL-6 in a variety of different assays. For example, the dissociation rate of IL-6 bound
20 to the ligand trap was measured in parallel with the dissociation rate of IL-6 from the anti-IL-6 monoclonal neutralizing antibody B-E8 [Brochier, et al., Int. J. Immunopharmacology 17:41-48 (1995), and references within]. An example of this type of experiment is shown in Figure 18. In this experiment 20 pM ¹²⁵I-IL-6 (1000 μ Ci/mmol; Amersham) was
25 preincubated with 500 pM of either gp130-C γ 1•IL-6R α - κ or mAb B-E8 for 20 hours. At this point a 1000-fold excess (20 nM) of "cold" IL-6 was added. Periodically, aliquots of the reaction were removed, the ligand trap or B-E8 were precipitated with Protein G-Sepharose, and the number of cpm of ¹²⁵I-IL-6 that remained bound was determined. Clearly, the dissociation
30 rate of human ¹²⁵I-IL6 from the ligand trap was very slow - after three days, approximately 75% of the initial counts were still bound to the ligand

trap. In contrast, less than 5% of the counts remained associated with the antibody after three days. This result demonstrates that the dissociation rate of the ligand from these ligand traps is very slow.

- 5 In a different set of experiments the ability of the ligand traps to multimerize in the presence of ligand was tested. An example of this is shown in Figure 19. IL-6-induced association of gp130-Fc•IL-6R α -Fc with gp130-CH1•IL-6R α - κ was determined by testing whether gp130-CH1•IL-6R α - κ , which does not by itself bind Protein A, could be precipitated by
- 10 Protein A-Sepharose in the presence of gp130-Fc•IL-6R α -Fc in an IL-6-depended manner (Figure 9). Precipitation of gp130-CH1•IL-6R α - κ by Protein A-Sepharose was determined by western blotting with an anti-kappa specific HRP conjugate, which does not detect gp130-Fc•IL-6R α -Fc. gp130-CH1•IL-6R α - κ could be precipitated by Protein A-Sepharose only
- 15 when both gp130-Fc•IL-6R α -Fc and IL-6 were present. This result conclusively indicates that IL-6 can induce ligand trap multimerization, and further indicate that the ligand trap can mimic the hexameric cytokine•R α •signal transducer complex (Figure 1). Ligand-induced multimerization may play a significant role in the clearance of
- 20 cytokine•ligand trap complexes *in vivo*.

- The biological activity of the different ligand traps may be further tested in assays which measure ligand-depended cell proliferation. Several cell proliferation assays exist for IL-6 and they employ cell lines such as B9,
- 25 CESS, or XG-1. An example of this type of assay using the XG-1 cell line is presented below: XG-1 is a cell line derived from a human multiple myeloma (Zhang, et al., Blood 83:3654-3663 (1994). XG-1 depends on exogenously supplied human IL-6 for survival and proliferation. The EC₅₀ of IL-6 for the XG-1 line is approximately 50 pmoles/ml. The ability of
- 30 several different IL-6 traps to block IL-6-depended proliferation of XG-1

cells was tested by incubating increasing amounts of purified ligand traps with 50 pg/ml IL-6 in XG-1 cultures. The ligand traps which were tested had been expressed and purified by methods similar to those described above. All of the ligand traps tested were found to inhibit IL-6-dependent proliferation of XG-1 in a dose dependent manner (Figure 20). Of the five different traps tested gp130-C γ 1•IL-6R α - κ was the most active and essentially display the same neutralizing activity towards IL-6 as the antibody B-E8. As little as a 10-fold molar excess of either gp130-C γ 1•IL-6R α - κ or B-E8 completely blocked the activity of IL-6 (a reading of A570-650 = 0.3 AU corresponds to no proliferation of the XG-1 cells). At a 100-fold molar excess all of the ligand traps tested completely blocked the activity of IL-6. This observed inhibition is highly selective as neither a gp130-Fc•CNTFR α -Fc ligand trap which blocks CNTF activity, nor gp130-Fc homodimer exhibit any blocking activity towards IL-6 even when used at a 1000-fold molar excess over IL-6 (data not shown). This data demonstrates that the heteromeric immunoglobulin heavy/light chain receptor-based ligand traps function as selective high affinity antagonists of their cognate ligand.

20 EXAMPLE 5 - CLONING OF FUSION POLYPEPTIDE COMPONENTS

The extracellular domains of the human cytokine receptors were obtained by standard PCR techniques using tissue cDNAs (CLONTECH), cloned into the expression vector, pMT21 (Genetics Institute, Inc.), and the sequences were sequenced by standard techniques using an ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA). For the IL-4R α , nucleotides 241 through 868 (corresponding to the amino acids 24-231) from the Genbank sequence, X52425, were cloned. For the IL-2R γ , nucleotides 15 through 776 (corresponding to amino acids 1-233) from the Genbank sequence, D11086, were cloned. For the IL-6R α , nucleotides 52 through 1044 (corresponding

to the amino acids 1-331) from the Genbank sequence, X52425, were cloned. For gp130, nucleotides 322 through 2112 (corresponding to the amino acids 30-619) from the Genbank sequence, M57230, were cloned. For the IL-1RAcP, nucleotides 1 through 1074 (corresponding to the amino acids 1-358) from the Genbank sequence, AB006357, were cloned. For the IL-1RI, nucleotides 55 through 999 (corresponding to the amino acids 19-333) from the Genbank sequence, X16896, were cloned.

10 EXAMPLE 6 - PRODUCTION OF FUSION POLYPEPTIDES (CYTOKINE TRAPS)

The nucleotide sequences encoding the cytokine traps were constructed from the individual cloned DNAs (described *supra*) by standard cloning and PCR techniques. In each case, the sequences were constructed in frame such that the sequence encoding the first fusion polypeptide component was fused to the sequence encoding the second fusion polypeptide component followed by an Fc domain (hinge, CH2 and CH3 region of human IgG1) as the multimerizing component. In some cases extra nucleotides were inserted in frame between sequences encoding the first and second fusion polypeptide components to add a linker region between the two components (See Figure 21A - Figure 21D - trap 424; Figure 24A - Figure 24F - trap 412; and Figure 26A - Figure 26E - trap 569).

For the IL-4 traps, 424 (Figure 21A - Figure 21D), 603 (Figure 22A - Figure 22D) and 622 (Figure 23A - Figure 23D), the IL-2R γ component is 5', followed by the IL4R α component and then the Fc component. For the IL-6 traps, 412 (Figure 24A - Figure 24F) and 616 (Figure 25A - Figure 25F), the IL-6R α component is 5' followed by the gp130 component and then the Fc domain. For the IL-1 trap 569 (Figure 26A - Figure 26E), the IL-1RAcP component is 5' followed by the IL-1RI component and then the Fc domain. The final constructs were cloned into the mammalian expression vector pCDNA3.1 (STRATAGENE).

In the 569 sequence (Figure 26A - Figure 26E), nucleotides 1-1074 encode the IL1RAcP component, nucleotides 1075 -1098 encode a linker region, nucleotides 1099-2043 encode the IL1RI component and nucleotides 2044-
5 2730 encode the Fc domain.

In the 412 sequence (Figure 24A - Figure 24F), nucleotides 1-993 encode the IL6R α component, nucleotides 994-1023 encode a linker region, nucleotides 1024-2814 encode the gp130 component and nucleotides 2815-
10 3504 encode the Fc domain.

In the 616 sequence (Figure 25A - Figure 25F), nucleotides 1-993 encode the IL6R α component, nucleotides 994-2784 encode the gp130 component and nucleotides 2785-3474 encode the Fc domain.
15

In the 424 (Figure 21A - Figure 21D) and 622 (Figure 23A - Figure 23D) sequences, nucleotides 1-762 encode the IL2R γ component, nucleotides 763-771 encode a linker region, nucleotides 772-1395 encode the IL4R α component and nucleotides 1396-2082 encode the Fc domain.
20

Finally, in the 603 sequence (Figure 22A - Figure 22D), nucleotides 1-762 encode the IL2R γ component, nucleotides 763-1386 encode the IL4R α component and nucleotides 1387-2073 encode the Fc domain.

25 DNA constructs were either transiently transfected into COS cells or stably transfected into CHO cells by standard techniques well known to one of skill in the art. Supernatants were collected and purified by Protein A affinity chromatography and size exclusion chromatography by standard techniques. (See for example Harlow and Lane, Antibodies - A Laboratory
30 Manual, Cold Spring Harbor Laboratory, 1988).

EXAMPLE 7: IL-4 BIOASSAY PROTOCOL USING TF-1 (ATCC) CELLS.Reagents and Equipment Needed5 MTT Dye Solution:

MTT(3-[4,5-Dimethylthiazole-2-yl]) (Sigma catalog# M2128)

Working concentration: Dissolve 5 mg of anhydrous MTT in 200 ml PBS without Ca^{+2} , Mg^{+2} .

10 Sterile filter and store aliquoted at -20°C

Solubilization Solution:

For 1000 ml, combine 100 g SDS, 950 ml dH_2O , 50 ml Dimethyl Formamide,
15 and 850 μl concentrated HCl.

Filter sterilize with a $0.45\mu\text{m}$ filter unit.

Store at room temperature

TF-1 cell Growth Medium:

20

RPMI 1640, 10% FBS, Pen/Strep, 2mM L-glutamine

Other:

25 0.4% Trypan Blue Stain, sterile tubes for dilutions, sterile 96 well cell culture plates (Falcon #3072), hemacytometer, centrifuge, ELISA plate reader, multichannel pipet for 15, 25, 50 and $100\mu\text{l}$ volume, sterile reagent reservoirs, sterile pipet tips, gloves.

Assay Protocol

A. Preparation of Assay plates

- 5 1. Prepare sterile 96 well tissue culture plates to contain 50µl of growth medium per well with various concentrations of IL-4 and 10nM IL-4 antagonist. This can be done by preparing a working dilution of IL-4 that is 4 times the highest concentration to be assayed. In separate tubes, do a two-fold serial dilution of the IL-4. Add 25µl of each dilution to one row
10 across the plate (i.e. row A gets highest concentration, row G gets lowest concentration). Add 25µl of growth medium without IL-4 to row H. Prepare the antagonists to be tested by making a stock that is 4 times the final concentration. Add 25µl to a triplicate set of IL-4 containing wells (columns 1,2,3, A through H). Be sure to include antagonist in row H.
15
2. As a positive control, leave one set with no antagonist. These wells will contain IL-4 and media only.
3. Incubate the plate for 1-2 hours at 37°C in a humidified 5% CO₂
20 incubator before preparing cells to be used for assay.

B. Preparation of Cells

- 25 4. Wash cells twice by centrifugation in assay medium free of growth factor.
5. Determine cell number and trypan blue viability and suspend cells to a final concentration of 8×10^5 /ml in assay medium.
- 30 6. Dispense 50µl of the cell suspension (40,000 cells) into all wells of the plates. Total volume should now be 100µl/well.

7. Incubate the plate at 37°C for 68 hours in a humidified 5% CO₂ incubator.

C. Color Development

5

8. After incubating for 68 hours, add 15μl of the MTT dye solution to each well.

9. Incubate the plate at 37°C for 4 hours in a humidified 5% CO₂ incubator.

10

10. After 4 hours, add 100μl of the solubilization solution to each well. Allow the plate to stand overnight in a sealed container to completely solubilize the formazan crystals.

15 11. Record the absorbance at 570/650nm.

RESULTS

Figure 27 shows that an IL-4 trap designated 4SC375, which is a fusion polypeptide of IL-2Rγ-scb-IL4Rα-FcΔC1, is several orders of magnitude better as an IL-4 antagonist than IL4RαFcΔC1 alone in the TF1 cell bioassay.

Figure 28 shows that the IL-4 trap designated 4SC375 shows antagonistic activity in the TF1 cell bioassay equivalent to an IL-4 trap designated 4SC424 which is a fusion polypeptide of IL-2Rγ-IL4Rα-FcΔC1 having the IL-2Rγ component flush with the IL-4Rα component.

EXAMPLE 8: IL-6 BIOASSAY PROTOCOL USING XG-1 CELLS

30 Reagents and Equipment Needed

MTT Dye Solution:

MTT(3-[4,5-Dimethylthiazole-2-yl]) (Sigma catalog# M2128)

Working concentration: Dissolve 5 mg of anhydrous MTT in 200 ml PBS

5 without Ca^{+2} , Mg^{+2} .

Sterile filter and store aliquoted at -20°C

Solubilization Solution:

10 For 1000 ml, combine 100 g SDS, 950 ml dH_2O , 50 ml Dimethyl Formamide, and 850 μl concentrated HCl.

Filter sterilize with at 0.45 μm filter unit.

Store at room temperature

15 Assay Medium:

RPMT 1640, 10%FBS, Pen/Strep, 2mM L-glutamine, 50 μM mercapto-ethanol.

20 Other:

0.4% Trypan Blue Stain, sterile tubes for dilutions, sterile 96 well cell culture plates (Falcon#3072), hemacytometer, centrifuge, ELISA plate reader, multichannel pipet for 15, 25, 50 and 100 μl volume, sterile reagent

25 reservoirs, sterile pipet tips, gloves.

Assay ProtocolA. Preparation of Assay plates

30

1. Prepare sterile 96 well tissue culture plates to contain 50 μl of growth medium per well with various concentrations of IL-6 and 10nM IL-6 antagonist. This can be done by preparing a working dilution of IL-6 that is

- 4 times the highest concentration to be assayed. In separate tubes, do a two-fold serial dilution of the IL-6. Add 25µl of each dilution to one row across the plate (i.e. row A gets highest concentration, row G gets lowest concentration). Add 25µl of growth medium without IL-6 to row H.
- 5 Prepare the antagonists to be tested by making a stock that is 4 times the final concentration. Add 25µl to a triplicate set of IL-6 containing wells (columns 1,2,3, A through H). Be sure to include antagonist in row H. A typical IL-6 titration starts at 200ng/ml down to 3.1ng/ml.
- 10 2. As a positive control, leave one set with no antagonist. These wells contain IL-6 and media in place of antagonist.
3. Incubate the plate 1-2 hours at 37°C in a humidified 5% CO₂ incubator before preparing cells to be used for assay.

15

B. Preparation of Cells

4. Wash cells twice by centrifugation (5 min at 1000RPM) in assay medium free of growth factor.
- 20 5. Determine cell number and trypan blue viability and suspend cells to a final concentration of 8×10^5 /ml in assay medium.
6. Dispense 50µl of the cell suspension (40000 cells) into all wells of the
- 25 plates. Total volume should now be 100µl/well.
7. Incubate the plate at 37°C for 68 hours in a humidified 5% CO₂ incubator.

C. Color Development

8. At 68 hours add 15µl of the dye solution to each well.

9. Incubate the plate at 37°C for 4 hours in a humidified 5% CO₂ incubator.
10. After 4 hours, add 100µl of the solubilization solution to each well.
Allow the plate to stand overnight in a sealed container to completely
5 solubilize the formazan crystals.
11. Record the absorbance at 570/650nm.

RESULTS

10

Figure 29 shows that the IL6 trap (6SC412 IL6R-scb-gpx-FcΔC1) described in Figure 24A - Figure 24F is a better antagonist of IL-6 in the XG1 bioassay than the neutralizing monoclonal antibody to human IL-6 - BE8.

15 EXAMPLE 9: MRC5 BIOASSAY FOR IL1 TRAPS

MRC5 human lung fibroblast cells respond to IL-1 by secreting IL-6 and thus were utilized to assay the ability of IL-1 traps to block the IL-1-dependent production of IL-6. IL1 Trap 1SC569 (Figure 26A - Figure 26E)
20 was tested against IL-1-RI.Fc which is the extracellular domain of the IL-1 Type I receptor fused to an Fc domain.

MRC5 cells are suspended at 1×10^5 cells per ml in medium and 0.1 ml of cells are plated (10,000 cells per well) into the wells of a 96 well tissue
25 culture plate. Plates are incubated for 24 hours at 37°C in a humidified 5% CO₂ incubator.

IL-1 trap and recombinant human IL-1 at varying doses are pre-incubated in a 96 well tissue culture dish and incubated for 2 hours at 37°C. 0.1 ml of
30 this mixture is then added to the 96 well plate containing the MRC5 cells such that the final concentration of IL-1 Trap is 10nM and the final

concentrations of the IL-1 ranges from 2.4 pM to 5nM. Control wells contain trap alone or nothing.

Plates are then incubated at 37°C for 24 hours in a humidified 5% CO₂ incubator. Supernatant is collected and assayed for levels of IL-6 using R&D Systems Quantikine Immunoassay Kit according to the manufacturer's instructions.

RESULTS

10

Figure 30 shows that the trap 569 (Figure 26A - Figure 26E) is able to antagonize the effects of IL-1 and block the IL-6 production from MRC 5 cells upon treatment with IL-1. At a concentration of 10nM, the trap 569 is able to block the production of IL-6 up to an IL-1 concentration of 3nM. In contrast, the IL-1RI.Fc is a much poorer antagonist of IL-1. It is only able to block the effects of IL-1 up to about 10-20 pM. Thus, the trap 569 is approximately 100x better at blocking IL-1 than IL1RI.Fc.

EXAMPLE 10 - CONSTRUCTION OF IL-13/IL-4 SINGLE CHAIN TRAPS

20

1. To create the IL-13/IL-4 dual trap designated IL-4R α .IL-13R α 1.Fc, the human IL-4R α extracellular domain (corresponding to nucleotides #1-693 of Figure 31A - Figure 31G) and the human IL-13R α 1 extracellular domain (corresponding to nucleotides #700-1665 of Figure 31A - Figure 31G) were amplified by standard PCR techniques and ligated into an expression vector pMT21 which contained the human Fc sequence (corresponding to nucleotides #1671-2355 of Figure 31A - Figure 31G), thus creating a fusion protein consisting of the IL-4R α , IL-13R α 1, and the hinge, CH2 and CH3 region of human IgG1 from the N to C terminus. In addition, a two amino acid linker (corresponding to nucleotides #694-699 of Figure 31A - Figure 31G) with the amino acid sequence SerGly was constructed in frame

between the IL-4R α and the IL-13R α 1 and a two amino acid linker (corresponding to nucleotides #1666-1671 of Figure 31A - Figure 31G) with the amino acid sequence ThrGly was constructed in frame between the IL-13R α 1 and the Fc portion. All sequences were sequence-verified by standard techniques. The IL-4R α .IL-13R α 1.Fc coding sequence was then subcloned into the expression vector pCDNA3.1 (Stratagene) using standard molecular biology techniques.

2. To create the IL-13/IL-4 dual trap designated IL-13R α 1.IL-4R α .Fc, the IL-13R α 1 extracellular domain (corresponding to nucleotides #1-1029 of Figure 32A - Figure 32G) and the human IL-4R α (corresponding to nucleotides # 1060-1692 of Figure 32A - Figure 32G) were amplified by standard PCR techniques and ligated into the expression vector pJFE14, which contains the human Fc sequence (corresponding to nucleotides #1699-2382 of Figure 32A - Figure 32G) to create a fusion protein consisting of the IL-13R α 1, IL-4R α , and the hinge, CH2 and CH3 region of human IgG1 from the N to C terminus. In addition, a ten amino acid linker with the amino acid sequence GlyAlaProSerGlyGlyGlyGlyArgPro (corresponding to nucleotide #1030-1059 of Figure 32A - Figure 32G) was constructed in frame between the IL-13R α 1 and the IL-4R α and a two amino acid linker (corresponding to nucleotides #1693-1698 of Figure 32A - Figure 32G) with the amino acid sequence SerGly was constructed in frame between IL-4R α and the Fc portion. All sequences were sequence-verified using standard techniques. The coding sequence of IL-13R α 1.IL-4R α .Fc was then subcloned into the expression vector pCDNA3.1 (Stratagene) using standard molecular biology techniques.

EXAMPLE 11: EXPRESSION OF IL-4R α .IL-13R α 1.Fc AND IL-13R α 1.IL-4R α .Fc

Large scale (1L) cultures of the pCAE801 (the DNA vector construct encoding IL-4R α .IL-13R α 1.Fc) and pCAE802 (the DNA plasmid construct encoding IL-13R α 1.IL-4R α .Fc) in DH10B cells were grown overnight in LB + ampicillin and the plasmid DNA was extracted using a Qiagen Endofree
5 Mega Kit following the manufacturer's protocol. The concentration of the purified plasmid DNA was determined in a UV spectrophotometer and fluorometer. The plasmid DNA was also verified by digestion of aliquots with BbsI, XmnI and NcoI restriction enzymes. All restriction enzyme digest fragments corresponded to the predicted sizes in a 1% agarose gel.
10
Forty 15 cm petri plates were seeded with CHO-K1/E1A cells at a density of 4×10^6 cells/plate. Plating media was Gibco Ham's F-12 w/10% Hyclone Fetal Bovine Serum (FBS) + penicillin/streptomycin and supplemented with glutamine. The following day each plate was transfected with 6 μ g of
15 pCAE801, or pCAE802, using Gibco Optimem and Gibco Lipofectamine in 12 ml volume, following the manufacturer's protocol. Four hours after adding the transfection mix to the cells 12 ml/plate of Optimem w/ 10% FBS was added. Plates were incubated at 37°C in a 5% CO₂ incubator overnight. The following day the media was removed from each plate
20 and 25 ml expression media (Gibco CHO-S-SFM II w/ glutamine + 1mM sodium butyrate) was added. The plates were incubated at 37°C for 3 days.

After 3 days of incubation the media was removed from each plate and centrifuged at 400 rpm in a swinging bucket rotor to pellet cells. The
25 supernatant was decanted into sterile 1L bottles and expressed protein was purified as described *infra*.

EXAMPLE 12: PURIFICATION OF IL-4R α .IL-13R α 1.Fc AND IL-13R α 1.IL-4R α .Fc PROTEIN FROM CULTURE MEDIA

30

1. Purification of IL-4R α .IL-13R α 1.Fc.

Human IL-4R α .IL-13R α 1.Fc was transiently expressed in CHO cells and supernatants were harvested from plate transfections as described *supra*.

5 Expression of the secreted protein was determined by a sandwich ELISA using goat anti-hIgG (γ chain specific; Sigma 1-3382) and goat anti-hIgG (Fc specific)-FITC conjugate (Sigma F9512) capture and report antibodies, respectively. The yield ranged from 5.8 to 9.2 mg (average of 7.5 mg) per liter of conditioned media. CompleteTM protease inhibitor tablets (Roche

10 Diagnostics Corp.) were dissolved into the media (1 tablet/L). The conditioned media was sterile filtered (0.22 μ m pore size) prior to loading onto a pre-equilibrated, 5 mL HiTrap[®] Protein A affinity column (Amersham Pharmacia Biotech) in Dulbecco's PBS buffer (Life Technologies), pH 7.4 at 4°C. The flow rate was ~1-2 mL/min. The

15 column was extensively washed with PBS buffer to remove nonspecifically bound proteins from the column. IL-4R α .IL-13R α 1.Fc was eluted using 20 mM sodium citrate, 150 mM NaCl, pH 3.5. The eluate was immediately neutralized by titrating with 1 M Tris-OH. The fractions containing protein were pooled and immediately dialyzed in PBS buffer,

20 pH 7.4 at 4°C. The recovery from Protein A purification was 6.8 mg (73%). IL-4R α .IL-13R α 1.Fc was further purified by size exclusion chromatography using a superose 6 column (25 mL bed volume; Amersham Pharmacia Biotech) pre-equilibrated in PBS, 5% v/v glycerol, pH 7.4 at ambient temperature. The flow rate was 0.5 mL/min. Protein fractions were

25 assessed from a Coomassie stained non-reduced and reduced SDS-PAGE (Novex NuPAGE 4-12% Bis-Tris gels). Fractions were conservatively pooled to reduce the amount of aggregated protein. The overall yield was 51% (4.4 mg) with a purity of 97% as judged by SDS-PAGE. Purified IL-4R α .IL-13R α 1.Fc was analyzed by non-reduced and reduced SDS-PAGE (4-

30 12% Bis-Tris), analytical size exclusion chromatography (Tosohaas

TSKG4000SWXL), N-terminal sequencing, and immunoblotting with goat anti-hIgG-HRP conjugate (Promega W403B), and also mouse monoclonal anti-hIL-4R (R&D MAB230) followed by anti-mIgG-HRP conjugate (Promega W402B) as the secondary antibody.

5

2. Purification of IL-13R α 1.IL-4R α .Fc

Human IL-13R α 1.IL-4R α .Fc was transiently expressed in CHO cells and supernatants were harvested from plate transfections as described *supra*.

10 Expression of the secreted protein was determined by a sandwich ELISA using goat anti-hIgG (γ chain specific; Sigma 1-3382) and goat anti-hIgG (Fc specific)-FITC conjugate (Sigma F9512) capture and report antibodies, respectively. The yield was 8.8 mg per liter of conditioned media. CompleteTM protease inhibitor tablets (Roche Diagnostics Corp.) were

15 dissolved into the media (1 tablet/L). The conditioned media was sterile filtered (0.22 μ m pore size) prior to loading onto a pre-equilibrated, 5 mL HiTrap[®] Protein A affinity column (Amersham Pharmacia Biotech) in Dulbecco's PBS buffer (Life Technologies), pH 7.4 at 4°C. The flow rate was ~1-2 mL/min. The column was extensively washed with PBS buffer to

20 remove nonspecifically bound proteins from the column. IL-13R α 1.IL-4R α .Fc was eluted using 20 mM sodium citrate, 150 mM NaCl, pH 3.5. The eluate was immediately neutralized by titrating with 1 M Tris-OH. The fractions containing protein were pooled and immediately dialyzed in PBS buffer, pH 7.4 at 4 °C. The recovery from Protein A purification was 3.8 mg

25 (43%). IL-13R α 1.IL-4R α .Fc was further purified by size exclusion chromatography using a superose 6 column (25 mL bed volume; Amersham Pharmacia Biotech) pre-equilibrated in PBS, 5% v/v glycerol, pH 7.4 at ambient temperature. The flow rate was 0.5 mL/min. Protein fractions were assessed from a Coomassie stained non-reduced and

30 reduced SDS-PAGE (Novex NuPAGE 4-12% Bis-Tris gels). Fractions were

conservatively pooled to reduce the amount of aggregated protein. The overall yield was 17% (1.5 mg) with a purity of 95% as judged by SDS-PAGE. Purified IL-13R α 1.IL-4R α .Fc was analyzed by non-reduced and reduced SDS-PAGE (4-12% Bis-Tris), analytical size exclusion chromatography (Tosohaas TSKG4000SWXL), N-terminal sequencing, and immunoblotting with goat anti-hIgG-HRP conjugate (Promega W403B), and also mouse monoclonal anti-hIL-4R α (R&D MAB230) followed by anti-mIgG-HRP conjugate (Promega W402B) as the secondary antibody.

10 EXAMPLE 13: BLOCKING OF IL-4 AND IL-13 BY IL-4R α .IL-13R α 1.Fc AND IL-13R α 1.IL-4R α .Fc

Materials and Methods

15 TF1 Bioassay. TF1 cells were maintained in growth media (10ng/ml GM-CSF, RPMI 1640, 10% FBS, L-glutamine, Penicillin, Streptomycin). For the bioassay, cells were washed 2 times in assay media (as above but without GM-CSF) and then plated at 2×10^5 cells in 50 μ l of assay media. The purified IL-4R α .IL-13R α 1.Fc and IL-13R α 1.IL-4R α .Fc proteins were diluted
20 into assay media at a concentration of 40nM. 25 μ l of each of the traps was added to the cells. Either IL-13 or IL-4 were diluted to 40nM in assay media and then 2-fold dilution series in assay media were made. 25 μ l of either IL-13 or IL-4 was then added to the wells containing the cells and the traps. Cells were then incubated at 37°C, 5% CO₂ for ~70 hrs. The extent of TF1
25 cell proliferation was measured by the MTS assay according to the manufacturer's protocol (Promega, Inc.).

RESULTS

30 The ability of the IL-4R α .IL-13R α 1.Fc and IL-13R α 1.IL-4R α .Fc traps to block both human IL-13 and human IL-4 activity was measured in the TF1

bioassay described *supra*. IL-13 stimulates proliferation of TF1 cells, with half-maximal growth at a concentration of 0.2nM. Addition of either IL-4R α .IL-13R α 1.Fc or IL-13R α 1.IL-4R α .Fc trap at a concentration of 10nM blocks IL-13-induced growth up to ~2nM (Figure 33). At an IL-13 concentration of ~4-5 nM the growth of TF1 cells is inhibited by 50%. TF1 cells are more sensitive to IL-4, which stimulates their proliferation with half-maximal growth at ~0.02nM. Addition of either IL-4R α .IL-13R α 1.Fc or IL-13R α 1.IL-4R α .Fc at a concentration of 10nM blocks IL-4-induced growth up to ~1nM (Figure 34). At an IL-4 concentration of ~3-4 nM the growth of TF1 cells is inhibited by 50%. These results show that both IL-4R α .IL-13R α 1.Fc and IL-13R α 1.IL-4R α .Fc can block the ability of both IL-13 and IL-4 to stimulate cellular responses.

EXAMPLE 14: BLOCKING OF INJECTED IL-1 BY IL-1 TRAP *IN VIVO*

IL-1 is a pro-inflammatory cytokine. Systemic administration of IL-1 has been shown to elicit acute responses in animals, including transient hyperglycemia, hypoinsulinemia, fever, anorexia, and increased serum levels of interleukin-6 (IL-6) (Reimers, 1998). Since mice are responsive to both murine and human IL-1, human IL-1 can be used and *in vivo* binding effects of human specific IL-1 antagonists can be evaluated. This acute mouse model was used to determine the ability of a human IL-1 trap to antagonize the *in vivo* effects of exogenously administered human IL-1. This provides a rapid indication of *in vivo* efficacy of the human IL-1 trap and can be used as an assay to help molecule selection.

Experimental Design:

Mice were given subcutaneous injections of human IL-1 (0.3 μ g/kg). Twenty-four hours prior to human IL-1 injection, the animals were pre-treated with either vehicle or 150-fold molar excess of human IL-1 trap (0.54 mg/kg). Two hours prior to sacrifice (26 hrs), the mice were given a

second injection of human IL-1 (0.3 µg/kg). Blood samples were collected at various time points and sera were assayed for IL-6 levels.

RESULTS

5

Exogenous administration of human IL-1 resulted a dramatic induction of serum IL-6 levels. At 150-fold molar excess, the human IL-1 trap completely blocked the IL-6 increase (Figure 35). Furthermore, the effects of the human IL-1 trap persisted for at least another 24 hours, preventing an IL-6 increase even when IL-1 was re-administered (Figure 35). Such long-lasting efficacy suggests that daily injection of an IL-1 trap may not be necessary for chronic applications.

EXAMPLE 15: EVALUATING THE ABILITY OF AN IL-4 TRAP TO BLOCK THE PHYSIOLOGICAL RESPONSES TO HUMAN IL-4 IN CYNOMOLOGUS MONKEYS.

Systemic administration of human IL-4 elicits systemic responses in Cynomologus monkeys (Gundel et al., 1996). Thus, the effectiveness of the IL-4 trap in blocking human IL-4 can be demonstrated by measuring these responses.

Experimental Design:

The experiment consisted of 3 parts: human IL-4 + vehicle (part 1), human IL-4 + IL-4 Trap (part 2), and human IL-4 + vehicle (part 3). Human IL-4 (25 µg/kg) was injected subcutaneously twice daily for 4 days and IL-4 Trap (8 mg/kg) and vehicle were given intravenously daily for 5 days, beginning 1 day prior to human IL-4 administration. Whole blood was collected daily for flow cytometry analysis for CD16 and plasma was obtained to assay for the cytokine monocyte chemotactic protein 1 (MCP-1).

CD16 and MCP-1 are markers of IL-4-mediated inflammation in both humans and monkeys.

RESULTS

5

In the presence of human IL-4, MCP-1 increased 2.5-fold and was significantly blocked by the IL-4 Trap (Figure 36A). Similarly, the decrease in the percent of CD16 positive lymphocytes in peripheral blood was attenuated by the IL-4 trap (Figure 36B). After a rest period, the monkeys
10 were re-injected with human IL-4 and the responsiveness of the animals to human IL-4 was re-confirmed (Figures 36A and 36B), suggesting that inhibition of the MCP-1 and CD 16 responses is specifically mediated by the IL-4 trap.

15 EXAMPLE 16: THE EFFECTS OF IL-4 TRAP ON IL-4-INDUCED IgE SECRETION.

It has been shown that injection of anti-mouse IgD antibody stimulates an IL-4-mediated IgE increase in normal mice. This model has been widely
20 used to evaluate IL-4 antagonists, such as soluble IL-4 receptor and anti-IL-4 monoclonal antibodies (Sato et al., 1993). We decided to use this model to evaluate the ability if the IL-4 trap to block IL-4-mediated increases of IgE.

25 Experimental design:

BALB/C mice injected with anti-mouse IgD (100µl/mouse, s.c.) were randomly divided into 3 groups. Each received (on days 3-5) either vehicle, murine IL-4 trap (1 mg/kg, s.c.), or a monoclonal antibody to
30 mouse IL-4 (1 mg/kg, s.c.). Serum was collected at various time points and assayed for IgE levels.

RESULTS

Treatment with the murine IL-4 trap or the mouse IL-4 antibody both significantly antagonized the IL-4-mediated IgE increase in this mouse
5 model (Figure 37). This suggests that the murine IL-4 trap binds murine IL-4 and antagonizes physiological responses elicited by endogenous IL-4 *in vivo*.

The present invention is not to be limited in scope by the specific
10 embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

15

WE CLAIM:

1. An isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex
5 comprising:
 - a) a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the specificity determining component of the cytokine's receptor;
 - 10 b) a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the signal transducing component of the cytokine's receptor; and
 - c) a nucleotide sequence encoding a third fusion polypeptide
15 component comprising the amino acid sequence of a multimerizing component.
2. The nucleic acid molecule of claim 1, wherein the nucleotide sequence encoding the first component is upstream of the nucleotide
20 sequence encoding the second component.
3. The nucleic acid molecule of claim 1, wherein the nucleotide sequence encoding the first component is downstream of the nucleotide sequence encoding the second component.
25
4. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the hematopoietin family of cytokines selected from the group consisting of interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-9,
30 interleukin-11, interleukin-13, interleukin-15, granulocyte macrophage colony stimulating factor, oncostatin M, and leukemia inhibitory factor and cardiotrophin-1

5. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the interferon family of cytokines selected from the group consisting of IFN-gamma, IFN-alpha, and IFN-beta.
- 5 6. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the immunoglobulin superfamily of cytokines selected from the group consisting of B7.1 (CD80) and B7.2 (B70).
- 10 7. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the TNF family of cytokines selected from the group consisting of TNF-alpha, TNF-beta, LT-beta, CD40 ligand, Fas ligand, CD 27 ligand, CD 30 ligand, and 4-1BBL.
- 15 8. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the TGF- β /BMP family selected from the group consisting of TGF- β 1, TGF- β 2, TGF- β 3, BMP-2, BMP-3a, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8a, BMP-8b, BMP-9, BMP-10, 20 BMP-11, BMP-15, BMP-16, endometrial bleeding associated factor (EBAF), growth differentiation factor-1 (GDF-1), GDF-2, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-12, GDF-14, mullerian inhibiting substance (MIS), activin-1, activin-2, activin-3, activin-4, and activin-5.
- 25 9. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a cytokine selected from the group consisting of interleukin-1, interleukin-10, interleukin-12, interleukin-14, interleukin-18 and MIF.
- 30 10. The isolated nucleic acid molecule of claim 1, wherein the multimerizing component comprises an immunoglobulin derived domain.

11. The isolated nucleic acid molecule of claim 10, wherein the immunoglobulin derived domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.
- 5 12. A fusion polypeptide encoded by the isolated nucleic acid molecule of claim 1.
13. A composition capable of binding a cytokine to form a nonfunctional complex comprising a multimer of the fusion polypeptide of claim 12.
- 10 14. The composition of claim 13, wherein the multimer is a dimer.
- 15 15. A vector which comprises the nucleic acid molecule of claim 1.
16. An expression vector comprising a nucleic acid molecule of claim 1, wherein the nucleic acid molecule is operatively linked to an expression control sequence.
- 20 17. A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 16, in a suitable host cell.
18. The host-vector system of claim 17, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell, or mammalian cell.
- 25 19. The host-vector system of claim 17, wherein the suitable host cell is E. coli.
- 30 20. The host-vector system of claim 17, wherein the suitable host cell is a COS cell.

21. The host-vector system of claim 17, wherein the suitable host cell is a CHO cell.
22. The host-vector system of claim 17, wherein the suitable host cell is
5 a 293 cell.
23. The host-vector system of claim 17, wherein the suitable host cell is a BHK cell.
- 10 24. The host-vector system of claim 17, wherein the suitable host cell is a NS0 cell.
25. A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claim 17, under conditions
15 permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.

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FIGURE 1

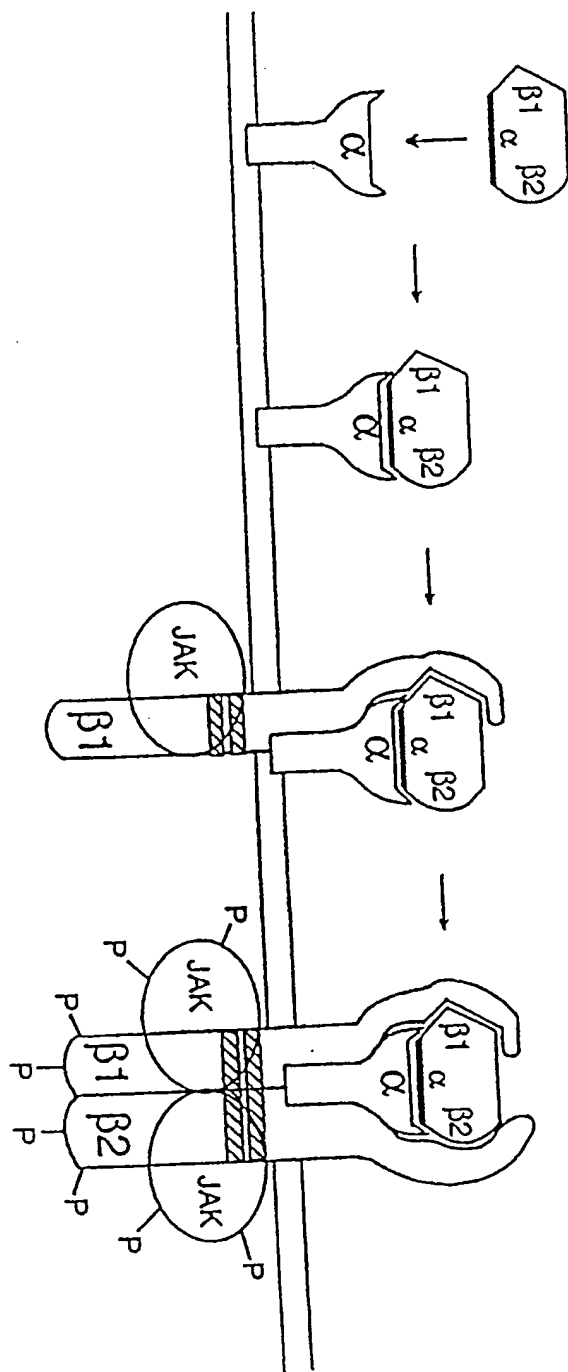
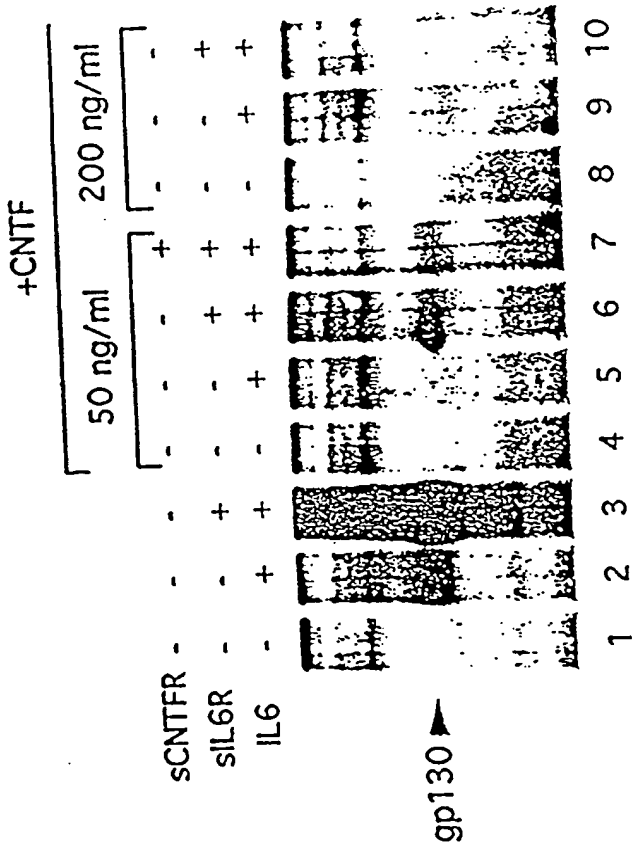


FIGURE 2



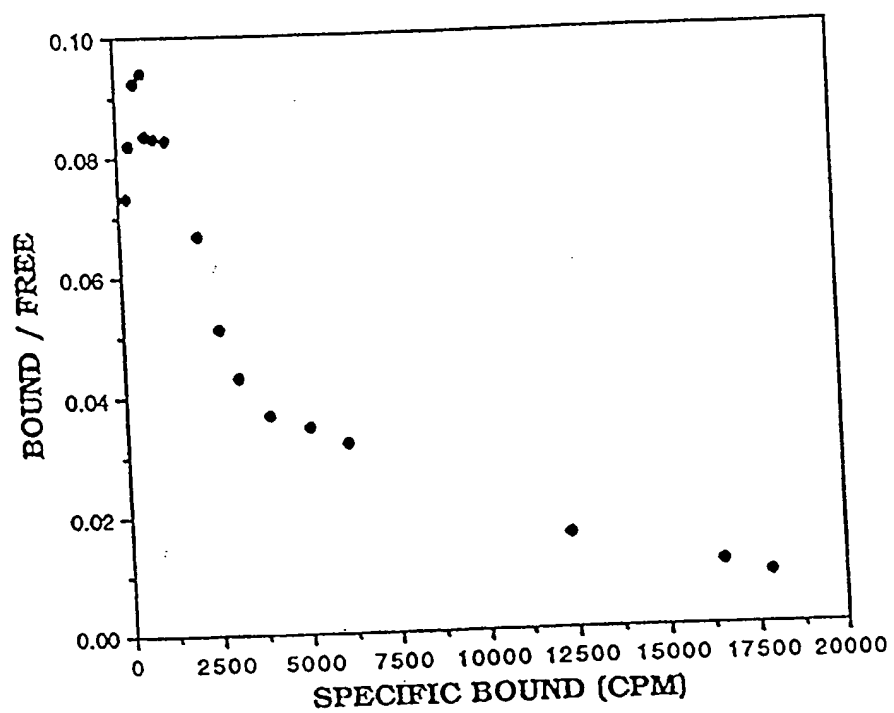


Figure 4

Amino acid sequence of human gp130-Fc-His6

Sequence Range: 1 to 861

10	20	30	40	50	60
*	*	*	*	*	*
MVTLQTVVQALFIFLT	TES	TGELLDP	CGYISP	ESPVVQ	LHSNFTAVCVLKEKCM
70	80	90	100	110	120
*	*	*	*	*	*
NANYIVWKT	NHFTIPKEQYT	IINRTASSVTFT	DIASLNIQ	LTCN	ILTFGQLEQNVYGITI
130	140	150	160	170	180
*	*	*	*	*	*
ISGLPPEKPK	NLS	SCIVNEGK	KMRCEWDGG	RETHLETNFTL	KSEWATHKFADCKAKRDTPT
190	200	210	220	230	240
*	*	*	*	*	*
SCTVDYSTVYFVN	IEVWVEA	ENALGKVTSD	HINFD	PVYKV	KPNPPHNLSVINSEELSSIL
250	260	270	280	290	300
*	*	*	*	*	*
KLTW	TNPSIKSVIILKYN	IQYRTKDA	STWSQIPPEDTAST	RSSFTVQDLKPFTEYVFRIR	
310	320	330	340	350	360
*	*	*	*	*	*
CMKEDGKGYWSDW	SEEASGI	TYEDRPSKAP	SFWYKIDPSH	TQGYRTVQLVWKTLP	PPFEAN
370	380	390	400	410	420
*	*	*	*	*	*
GKILDYEVTLTRW	KSHLQNY	TVNATKLT	VNLTNDRYLATL	TVRN	LVGKSDAAVLTIPACD
430	440	450	460	470	480
*	*	*	*	*	*
FQATHP	VMDLKAFPKDNMLW	VEWTT	PRESVKYILEWCVL	SDKAPCITDWQQEDG	TVHRT
490	500	510	520	530	540
*	*	*	*	*	*
YLRGNLAESKCYLIT	VTVPV	ADGPGSPESIKAYLKQAPPS	KGPTV	RTKKVGKNEAVLEWD	
550	560	570	580	590	600
*	*	*	*	*	*
QLPVDVQNGFIRNYTIF	YRT	IIGNETAVNV	DSSHTEYTLS	SLTSD	TYLMVRMAAYTDEGG
610	620	630	640	650	660
*	*	*	*	*	*
KDGPEFTFTTPKFAQGEIES	<u>GEPKSCDKTHTCPPCPAPEL</u>	<u>LGGPSVFLFPPKPKDTLMIS</u>			
670	680	690	700	710	720
*	*	*	*	*	*
RTPEVTCVVVDVSHEDPEVK	<u>FNWYVDGVEVHNAKTKPREE</u>	<u>OYNSTYRVVSVLTVLHODWL</u>			
730	740	750	760	770	780
*	*	*	*	*	*

FIGURE 4 continued

NGKEYKCKVSNKALPAIEK TISKAKGOPREPOVYTLPPS RDELTKNOVSLTCLVKGEYP

790

800

810

820

830

840

*

*

*

*

*

*

SDIAVEWESNGOPENNYKTT PPVLDSGDGSFFLYSKLTVDK SRWOOGNVFSCSVMHEALHN

850

860

*

*

HYTOKSLSLSPGKHHHHHH•

The amino acid sequence of human IL-6R α -Fc

Sequence Range: 1 to 594

10	20	30	40	50	60
*	*	*	*	*	*
MVAVGCALLAALLAAPGAAL	APRRCPAQEVARGVLTSLPG	DSVTLTCTPGVEPEDNATVHW			
70	80	90	100	110	120
*	*	*	*	*	*
VLRKPAAGSHPSRWAGMGRR	LLLRVQLHDSGNYSCYRAG	RPAGTVHLLVDVPPEEPQLS			
130	140	150	160	170	180
*	*	*	*	*	*
CFRKSPLSNVVCEWGRSTP	SLTTKAVLLVRKFQNSPAED	FQEPQYSQESQKFSCQLAV			
190	200	210	220	230	240
*	*	*	*	*	*
PEGDSSFYIVSMCVASSVGS	KFSKTQTFQCGILQPDPPA	NITVTAVARNPRWLSVTWQD			
250	260	270	280	290	300
*	*	*	*	*	*
PHSWNSSFYRLRFELRYRAE	RSKTFTTWVVKDLQHHCVIH	DAWSGLRHVVQLRAQEEFGQ			
310	320	330	340	350	360
*	*	*	*	*	*
GEWSEWSPEAMGTPWTESRS	PPAENEVSTPMQALTTNKDD	DNILFRDSANATSLPVQDAG			
370	380	390	400	410	420
*†	*†	*	*	*	*
EPKSCDKTHTCPPCPAPELL	GGPSVFLFPPKPKDTLMISR	TPEVTCVVVDVSHEDPEVKF			
430	440	450	460	470	480
*	*	*	*	*	*
NWYVDGVEVHNAKTKPREEO	YNSTYRVVSVLTVLHODWLN	GKEYCKVSNKALPAPIEKT			
490	500	510	520	530	540
*	*	*	*	*	*
ISKAKGPREPOVYTLPPSR	DELTKNOVSLTCLVKGFYPS	DIAVEWESNGOPENNYKTP			
550	560	570	580	590	
*	*	*	*	*	
PVLDSDGSFFLYSKLTVDKS	RWQOGNVFSCSVMHEALHNH	YTOKSLSLSPGK			

FIGURE 6

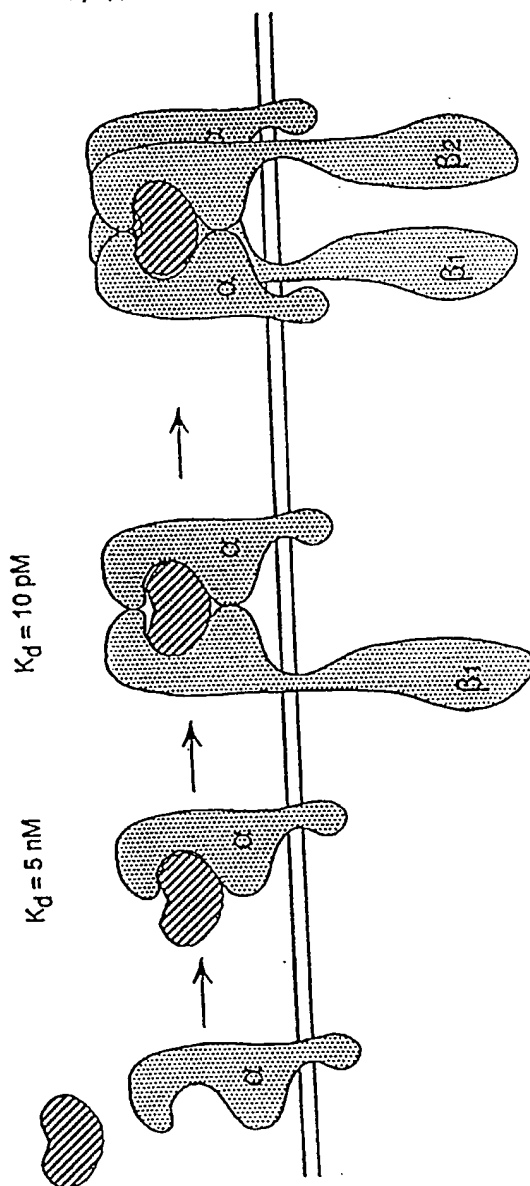


FIGURE 7
Heterodimeric Receptor-Based Ligand Trap

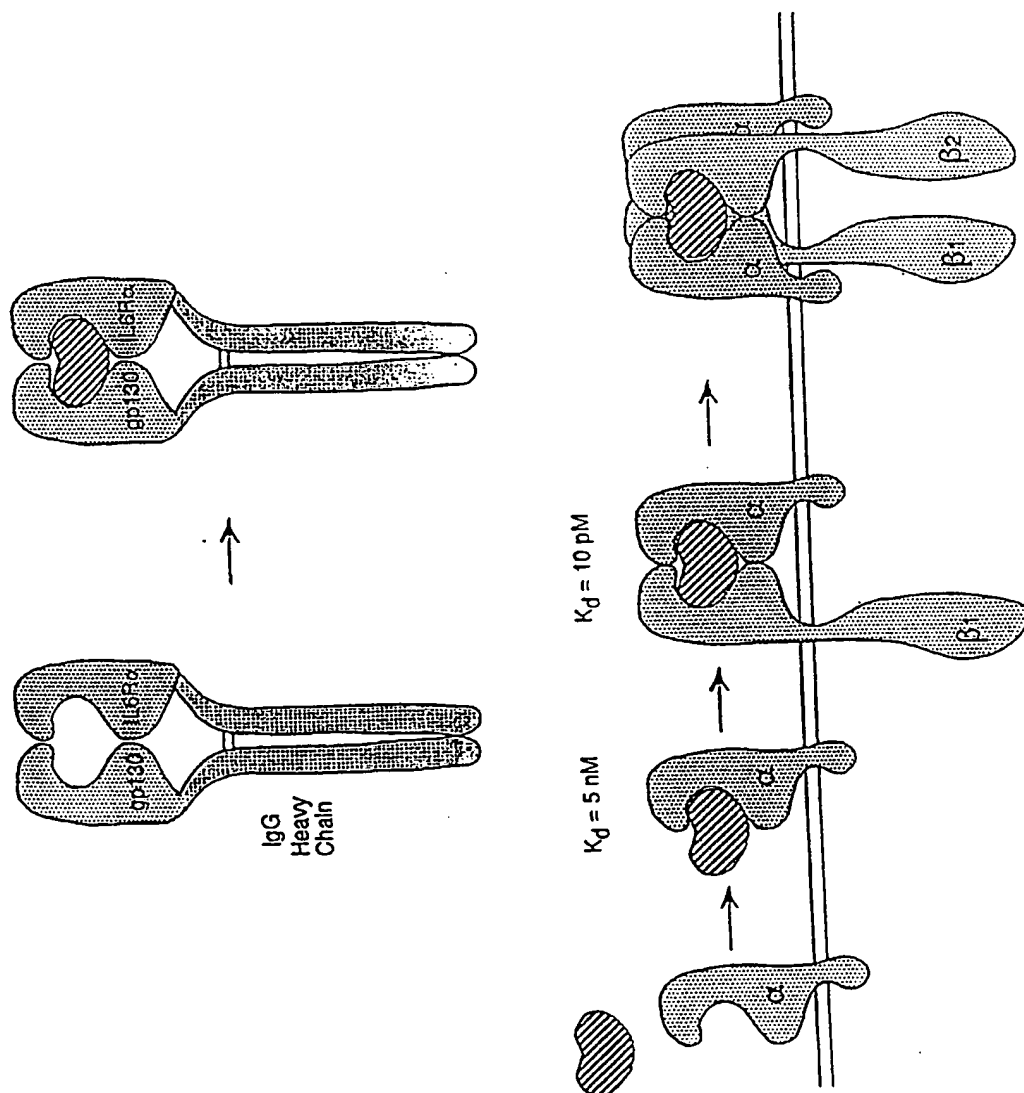
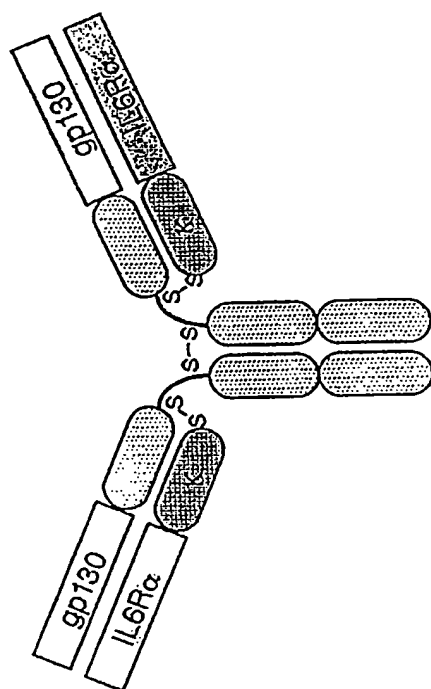


FIGURE 8

Immunoglobulin Heavy/Light Chain Receptor Fusions



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FIGURE 9

Amino acid sequence of gp130-Cy1

Sequence Range: 1 to 952

10	20	30	40	50	60
*	*	*	*	*	*
MVTLQTWVVQALFIFLTES	TGELLDPG	YISPE	SPVVQL	HSNFTAV	CVLKEKCM
70	80	90	100	110	120
*	*	*	*	*	*
NANYIVWKT	NHFTIPKEQYT	IINRTASSVT	FTDIASLNIQ	LTCNILT	FGQLEQNVYGITI
130	140	150	160	170	180
*	*	*	*	*	*
ISGLPPEKPKNL	SCIVNEGK	KMRCEWDG	GRETHLETNFTL	KSEWATHK	FADCKAKRDTPT
190	200	210	220	230	240
*	*	*	*	*	*
SCTVDYSTVYFVN	IEVWVEA	ENALGKVTSD	HINFDVPYKV	KPNPPHNLS	VINSEELSSIL
250	260	270	280	290	300
*	*	*	*	*	*
KLTWNP	SIKSVIILKYNIQ	YRTKDASTWS	QIPPEDTAST	RSSFTVQDL	KPFTEYVFRIR
310	320	330	340	350	360
*	*	*	*	*	*
CMKEDGKGYWSD	WSEEASGI	TYEDRPSKAPS	FWYKIDPSH	TQGYRTVQL	VWKTLPPEAN
370	380	390	400	410	420
*	*	*	*	*	*
GKILDYEV	TLTRWKSHLQNY	TVNATKLTV	NLTNDRYLATL	TVRNLVGKS	DAAVLTIPACD
430	440	450	460	470	480
*	*	*	*	*	*
FQATHPVM	DLKAFPKDNMLW	VEWTTPRES	VKKYILEWCVL	SDKAPCITD	WQQEDGTVHRT
490	500	510	520	530	540
*	*	*	*	*	*
YLRGNLAES	KCYLITVTPVY	ADGPGSPES	IKAYLKQAPPS	KGPTVVRT	KKVGKNEAVLEWD
550	560	570	580	590	600
*	*	*	*	*	*
QLPVDVQNG	FIRNYTIFYRT	IIGNETAVN	VDSSHTEYTLS	SLTSDTLYM	VRMAAYTDEGG
610	620	630	640	650	660
*	*	*	*	*	*
KDGPEFTFT	TPKFAQGEIES	GASTKGPSV	FPLAPSSKSTS	GGTAALGCL	VKDYEPEPVTV
670	680	690	700	710	720
*	*	*	*	*	*
SWNSGALTSG	VHTFPAVLOS	SGLYSLSSV	VTVPSSSLGTO	TYICNVNHK	PSNTKVDKKVE
730	740	750	760	770	780
*	*	*	*	*	*
PKSCDKTHTC	PPCPAPELLG	GPSVFLFP	KPKD	TLMI	SRT PEVTCVVVDVSHEDPEVKFN

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FIGURE 9 continued

790	800	810	820	830	840
*	*	*	*	*	*
<u>WYVDGVEVHNAKTKPREEOY NSTYRVVSVLTVLHODWLNG KEYKCKVSNKALPAPIEKTI</u>					
850	860	870	880	890	900
*	*	*	*	*	*
<u>SKAKGOPREPOVYTLPPSRD ELTKNOVSLTCLVKGFYPSD IAVEWESNGOPENNYKTPP</u>					
910	920	930	940	950	
*	*	*	*	*	
<u>VLDSGGSFFLYSKLTVDKSR WOOGNVFSCSVMHEALHNHY TOKSLSLSPGK*</u>					

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FIGURE 10

Amino acid sequence of gp130 Δ 3fibro

Sequence Range: 1 to 332

10	20	30	40	50	60
*	*	*	*	*	*
MVTLQTWVVQALFIFLT	TES	TGELLDPCGYISPES	PVVQL	HSNFTAVCVLKEKCM	DYFHV
70	80	90	100	110	120
*	*	*	*	*	*
NANYIVWKTNHFTIPKE	QYT	IINRTASSVTFTDIAS	LNIQ	LTCNILTFGQLEQN	VYGITI
130	140	150	160	170	180
*	*	*	*	*	*
ISGLPPEKPKNLSCIV	NEGK	KMRCEWDGGRETHLE	TNFTL	KSEWATHKFKADCKA	KRDTPT
190	200	210	220	230	240
*	*	*	*	*	*
SCTVDYSTVYFVNIEV	WVEA	ENALGKVTSDHINFDP	VYKV	KPNPPHNLSVINSE	ELSSIL
250	260	270	280	290	300
*	*	*	*	*	*
KLTWTNPSIKSVIILKY	NIQ	YRTKDASTWSQIPP	EDTAST	RSSFTVQDLKPFTE	YVFRIR
310	320	330			
*	*	*			
CMKEDGKGYWSDWSEE	ASGI	TYEDRPSKAPSG			

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FIGURE 11

Amino acid sequence of J-CH1

Sequence Range: 1 to 121

10	20	30	40	50	60
*	*	*	*	*	*
<u>SGGQGLVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTS</u>					
70	80	90	100	110	120
*	*	*	*	*	*
<u>GVHTFPAVLQSSGLYSLSSV VTPSSSLGTOTYICNVNHK PSNTKVDDKKVEPKSCDKTHT*</u>					

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FIGURE 12

Amino acid sequence of C γ 4

Sequence Range: 1 to 330

10	20	30	40	50	60
*	*	*	*	*	*
SGASTKGPSVFPLAPCSRST	SESTAALGCLVKDYFPEPVT	VS	WNSGALTSGVHTFPAVLQ		
70	80	90	100	110	120
*	*	*	*	*	*
SSGLYSLSSVVTVPSSSLGT	KTYTCNV	DHKPSNTKVDKRV	ESKYGPPCPSCP	PAPEFLGGP	
130	140	150	160	170	180
*	*	*	*	*	*
SVFLFPPKPKDTLMISRTPE	VTCVVVDVSQEDPEVQFNWY	VDGVEVHNAKTKPREEQFNS			
190	200	210	220	230	240
*	*	*	*	*	*
TYRVVSVLTVLHQDWLNGKE	YKCKVSNKGLPSSIEKTISK	AKGQPREPQVYTLPPSQEEM			
250	260	270	280	290	300
*	*	*	*	*	*
TKNQVSLTCLVKGFYPSDIA	VEWESNGQPENNYKTTPPVL	DSDGSFFLYSRLTVDKSRWQ			
310	320	330			
*	*	*			
EGNVFSCSVMEALHNHYTQ	KSLSLSLGK*				

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FIGURE 13

Amino acid sequence of κ -domain

Sequence Range: 1 to 108

10	20	30	40	50	60
*	*	*	*	*	*
SGTVAAPSVFIFPPSDEQLK	SGTASVVCLLNNFYPREAKV	QWKVDNALQSGNSQESVTEQ			
70	80	90	100		
*	*	*	*		
DSKDSTYSLSSTLTLSKADY	EKKHVYACEVTHQGLSSPVT	KSFNRGEC*			

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FIGURE 14

Amino acid sequence of λ -domain:

Sequence Range: 1 to 107

10	20	30	40	50	60
*	*	*	*	*	*
SGPKAAPSVTLFPPSSEELQ ANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTPSK					
70	80	90	100		
*	*	*	*		
QSNNKYAASSYLSLTPEQWK SHRSYSCQVTHEGSTVEKTV APTECS*					

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FIGURE 15

Amino acid sequence of the soluble IL-6 α domain

Sequence Range: 1 to 360

10	20	30	40	50	60
*	*	*	*	*	*
MVAVGCALLAALLAAPGAAL	APRRCPAQEVARGVLTSLPG	DSVTLTCPGVEPEDNATVHW			
70	80	90	100	110	120
*	*	*	*	*	*
VLRKPAAGSHPSRWAGMGRR	LLLRSVQLHDSGNYSYRAG	RPAGTVHLLVDVPPEEPQLS			
130	140	150	160	170	180
*	*	*	*	*	*
CFRKSPLSNVVCEWGPSTP	SLTTKAVLLVRKFQNSPAED	FQEPQYSQESQKFSCQLAV			
190	200	210	220	230	240
*	*	*	*	*	*
PEGDSSFYIVSMCVASSVGS	KFSKTQTFQCGILQPDPPA	NITVTAVARNPRWLSVTWQD			
250	260	270	280	290	300
*	*	*	*	*	*
PHSWNSSFYRLRFELRYRAE	RSKTFTTWMVKDLQHHCVIH	DAWSGLRHVVQLRAQEEFGQ			
310	320	330	340	350	360
*	*	*	*	*	*
GEWSEWSPEAMGTPWTESRS	PPAENEVSTPMQALTTNKDD	DNILFRDSANATSLPVQDAG			

FIGURE 16

Amino acid sequence of the soluble IL-6 α 313 domain

Sequence Range: 1 to 315

10	20	30	40	50	60
*	*	*	*	*	*
MVAVGCALLAALLAAPGAAL	APRRCPAQEVARGVLTSLPG	DSVTLTCPGVEPEDNATVHW			
70	80	90	100	110	120
*	*	*	*	*	*
VLRKPAAGSHPSRWAGMGRR	LLLRVQLHDSGNYSCYRAG	RPAGTVHLLVDVPPEEPQLS			
130	140	150	160	170	180
*	*	*	*	*	*
CFRKSPLSNVVCEWGPRSTP	SLTTKAVLLVRKFQNSPAED	FQEPQCYSQESQKFSCQLAV			
190	200	210	220	230	240
*	*	*	*	*	*
PEGDSSFYIVSMCVASSVGS	KFSKTQTFQCGILQPDPPA	NITVTAVARNPRWLSVTWQD			
250	260	270	280	290	300
*	*	*	*	*	*
PHSWNSSFYRLRFELRYRAE	RSKTFTTWMVKDLQHHCVIH	DAWSGLRHVVQLRAQEEFGQ			
310					
*					
GEWSEWSPEAMGTTG					

FIGURE 17

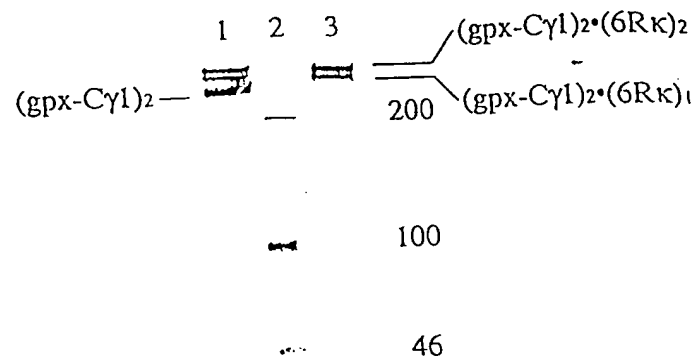


FIGURE 18

IL-6 Dissociates Slowly from the Ligand Trap

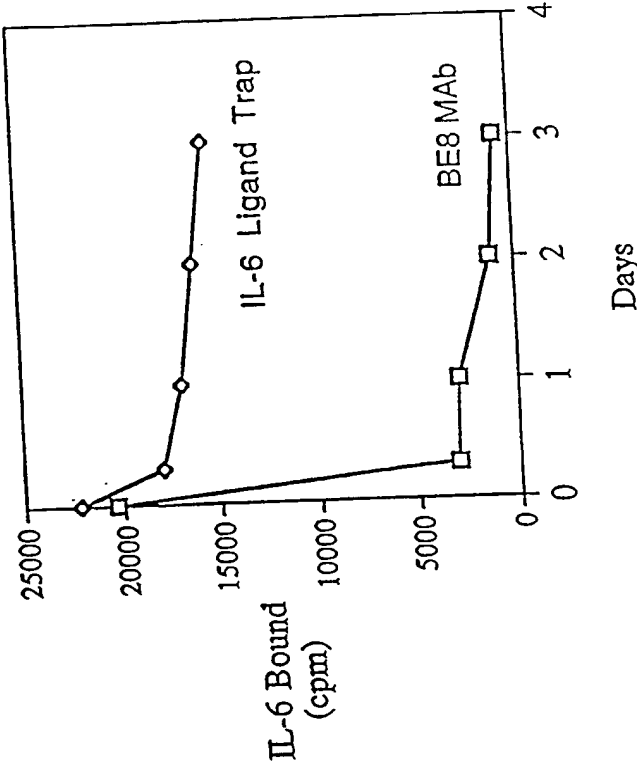


FIGURE 19

Does IL-6 Induce Complex Formation?

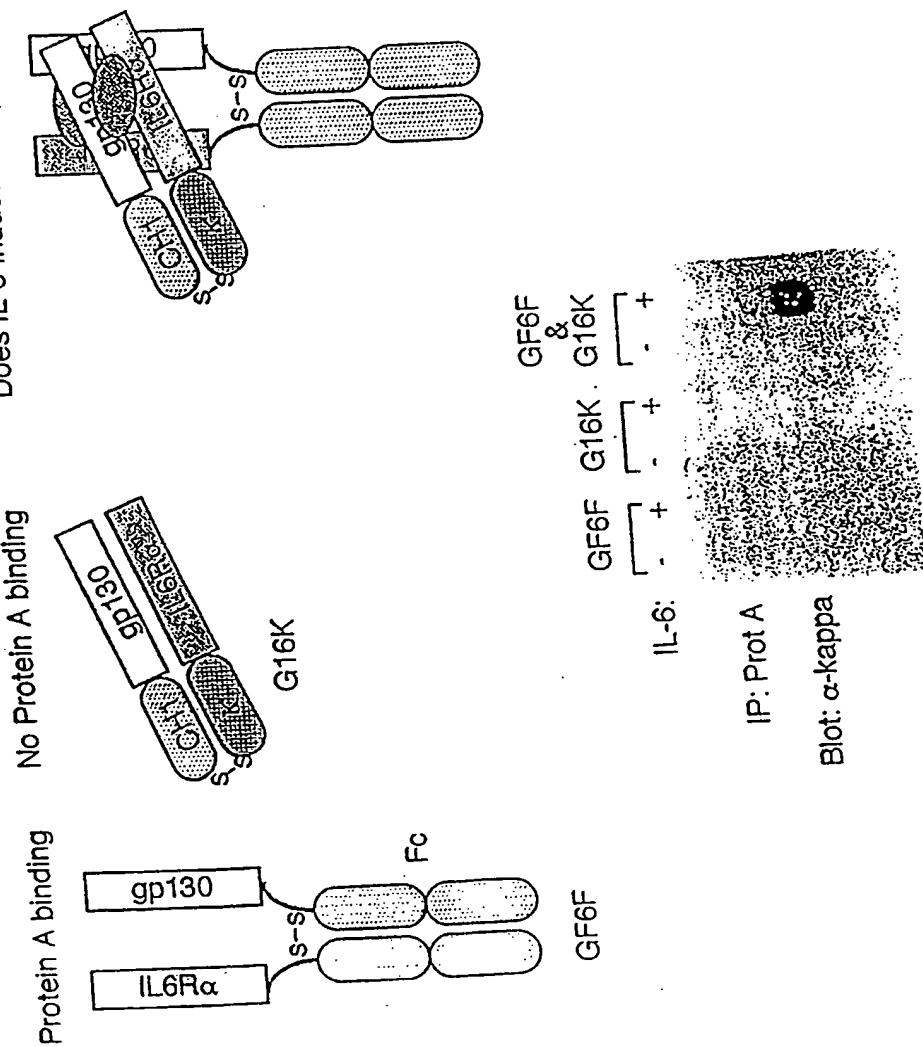
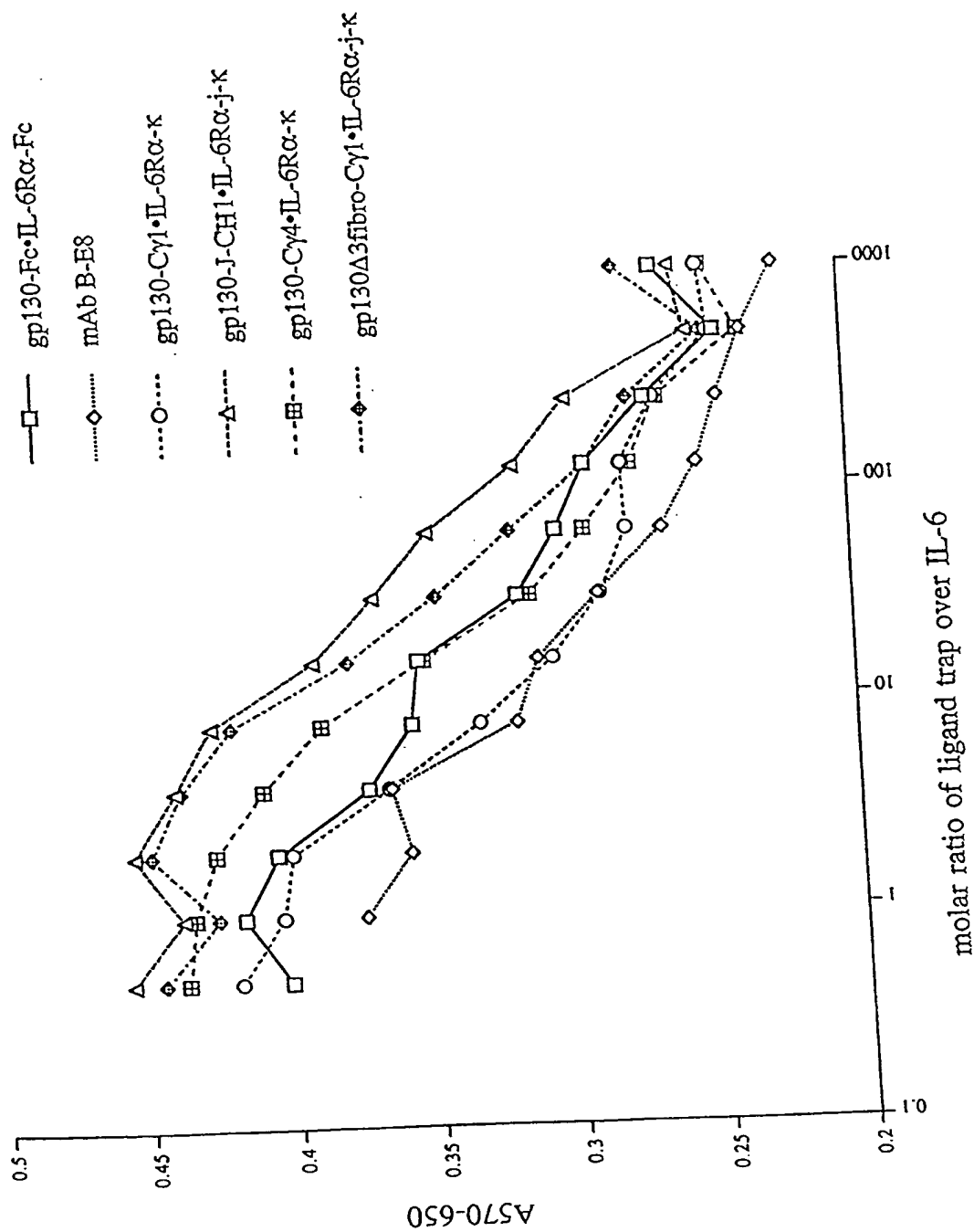


FIGURE. 20

XG-1 cell proliferation assay




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      10      20      30      40
      *      *      *      *
ATG GTG AAG CCA TCA TTA CCA TTC ACA TCC CTC TTA TTC CTG CAG CTG
Met Val Lys Pro Ser Leu Pro Phe Thr Ser Leu Leu Phe Leu Gln Leu>

50      60      70      80      90
      *      *      *      *      *
CCC CTG CTG GGA GTG GGG CTG AAC ACG ACA ATT CTG ACG CCC AAT GGG
Pro Leu Leu Gly Val Gly Leu Asn Thr Thr Ile Leu Thr Pro Asn Gly>

100     110     120     130     140
      *      *      *      *      *
AAT GAA GAC ACC ACA GCT GAT TTC TTC CTG ACC ACT ATG CCC ACT GAC
Asn Glu Asp Thr Thr Ala Asp Phe Phe Leu Thr Thr Met Pro Thr Asp>

150     160     170     180     190
      *      *      *      *      *
TCC CTC AGT GTT TCC ACT CTG CCC CTC CCA GAG GTT CAG TGT TTT GTG
Ser Leu Ser Val Ser Thr Leu Pro Leu Pro Glu Val Gln Cys Phe Val>

200     210     220     230     240
      *      *      *      *      *
TTC AAT GTC GAG TAC ATG AAT TGC ACT TGG AAC AGC AGC TCT GAG CCC
Phe Asn Val Glu Tyr Met Asn Cys Thr Trp Asn Ser Ser Ser Glu Pro>

250     260     270     280
      *      *      *      *
CAG CCT ACC AAC CTC ACT CTG CAT TAT TGG TAC AAG AAC TCG GAT AAT
Gln Pro Thr Asn Leu Thr Leu His Tyr Trp Tyr Lys Asn Ser Asp Asn>

290     300     310     320     330
      *      *      *      *      *
GAT AAA GTC CAG AAG TGC AGC CAC TAT CTA TTC TCT GAA GAA ATC ACT
Asp Lys Val Gln Lys Cys Ser His Tyr Leu Phe Ser Glu Glu Ile Thr>

340     350     360     370     380
      *      *      *      *      *
TCT GGC TGT CAG TTG CAA AAA AAG GAG ATC CAC CTC TAC CAA ACA TTT
Ser Gly Cys Gln Leu Gln Lys Lys Glu Ile His Leu Tyr Gln Thr Phe>

390     400     410     420     430
      *      *      *      *      *
GTT GTT CAG CTC CAG GAC CCA CGG GAA CCC AGG AGA CAG GCC ACA CAG
Val Val Gln Leu Gln Asp Pro Arg Glu Pro Arg Arg Gln Ala Thr Gln>

440     450     460     470     480
      *      *      *      *      *
ATG CTA AAA CTG CAG AAT CTG GTG ATC CCC TGG GCT CCA GAG AAC CTA
Met Leu Lys Leu Gln Asn Leu Val Ile Pro Trp Ala Pro Glu Asn Leu>

490     500     510     520
      *      *      *      *
ACA CTT CAC AAA CTG AGT GAA TCC CAG CTA GAA CTG AAC TGG AAC AAC
Thr Leu His Lys Leu Ser Glu Ser Gln Leu Glu Leu Asn Trp Asn Asn>

530     540     550     560     570
      *      *      *      *      *
AGA TTC TTG AAC CAC TGT TTG GAG CAC TTG GTG CAG TAC CGG ACT GAC
Arg Phe Leu Asn His Cys Leu Glu His Leu Val Gln Tyr Arg Thr Asp>

```

```

580          590          600          610          620
*           *           *           *           *
TGG GAC CAC AGC TGG ACT GAA CAA TCA GTG GAT TAT AGA CAT AAG TTC
Trp Asp His Ser Trp Thr Glu Gln Ser Val Asp Tyr Arg His Lys Phe>

630          640          650          660          670
*           *           *           *           *
TCC TTG CCT AGT GTG GAT GGG CAG AAA CGC TAC ACG TTT CGT GTT CGG
Ser Leu Pro Ser Val Asp Gly Gln Lys Arg Tyr Thr Phe Arg Val Arg>

680          690          700          710          720
*           *           *           *           *
AGC CGC TTT AAC CCA CTC TGT GGA AGT GCT CAG CAT TGG AGT GAA TGG
Ser Arg Phe Asn Pro Leu Cys Gly Ser Ala Gln His Trp Ser Glu Trp>

730          740          750          760
*           *           *           *           *
AGC CAC CCA ATC CAC TGG GGG AGC AAT ACT TCA AAA GAG AAC GCG TCG
Ser His Pro Ile His Trp Gly Ser Asn Thr Ser Lys Glu Asn Ala Ser>

770          780          790          800          810
*           *           *           *           *
TCT GGG AAC ATG AAG GTC CTG CAG GAG CCC ACC TGC GTC TCC GAC TAC
Ser Gly Asn Met Lys Val Leu Gln Glu Pro Thr Cys Val Ser Asp Tyr>

820          830          840          850          860
*           *           *           *           *
ATG AGC ATC TCT ACT TGC GAG TGG AAG ATG AAT GGT CCC ACC AAT TGC
Met Ser Ile Ser Thr Cys Glu Trp Lys Met Asn Gly Pro Thr Asn Cys>

870          880          890          900          910
*           *           *           *           *
AGC ACC GAG CTC CGC CTG TTG TAC CAG CTG GTT TTT CTG CTC TCC GAA
Ser Thr Glu Leu Arg Leu Leu Tyr Gln Leu Val Phe Leu Leu Ser Glu>

920          930          940          950          960
*           *           *           *           *
GCC CAC ACG TGT ATC CCT GAG AAC AAC GGA GGC GCG GGG TGC GTG TGC
Ala His Thr Cys Ile Pro Glu Asn Asn Gly Gly Ala Gly Cys Val Cys>

970          980          990          1000
*           *           *           *           *
CAC CTG CTC ATG GAT GAC GTG GTC AGT GCG GAT AAC TAT ACA CTG GAC
His Leu Leu Met Asp Asp Val Val Ser Ala Asp Asn Tyr Thr Leu Asp>

1010          1020          1030          1040          1050
*           *           *           *           *
CTG TGG GCT GGG CAG CAG CTG CTG TGG AAG GGC TCC TTC AAG CCC AGC
Leu Trp Ala Gly Gln Gln Leu Leu Trp Lys Gly Ser Phe Lys Pro Ser>

1060          1070          1080          1090          1100
*           *           *           *           *
GAG CAT GTG AAA CCC AGG GCC CCA GGA AAC CTG ACA GTT CAC ACC AAT
Glu His Val Lys Pro Arg Ala Pro Gly Asn Leu Thr Val His Thr Asn>

1110          1120          1130          1140          1150
*           *           *           *           *
GTC TCC GAC ACT CTG CTG CTG ACC TGG AGC AAC CCG TAT CCC CCT GAC
Val Ser Asp Thr Leu Leu Leu Thr Trp Ser Asn Pro Tyr Pro Pro GAC>

1160          1170          1180          1190          1200
*           *           *           *           *

```

AAT TAC CTG TAT AAT CAT CTC ACC TAT GCA GTC AAC ATT TGG AGT GAA
 Asn Tyr Leu Tyr Asn His Leu Thr Tyr Ala Val Asn Ile Trp Ser Glu>

1210 1220 1230 1240
 * * * * *
 AAC GAC CCG GCA GAT TTC AGA ATC TAT AAC GTG ACC TAC CTA GAA CCC
 Asn Asp Pro Ala Asp Phe Arg Ile Tyr Asn Val Thr Tyr Leu Glu Pro>

1250 1260 1270 1280 1290
 * * * * *
 TCC CTC CGC ATC GCA GCC AGC ACC CTG AAG TCT GGG ATT TCC TAC AGG
 Ser Leu Arg Ile Ala Ala Ser Thr Leu Lys Ser Gly Ile Ser Tyr Arg>

1300 1310 1320 1330 1340
 * * * * *
 GCA CGG GTG AGG GCC TGG GCT CAG TGC TAT AAC ACC ACC TGG AGT GAG
 Ala Arg Val Arg Ala Trp Ala Gln Cys Tyr Asn Thr Thr Trp Ser Glu>

1350 1360 1370 1380 1390
 * * * * *
 TGG AGC CCC AGC ACC AAG TGG CAC AAC TCC TAC AGG GAG CCC TTC GAG
 Trp Ser Pro Ser Thr Lys Trp His Asn Ser Tyr Arg Glu Pro Phe Glu>

1400 1410 1420 1430 1440
 * * * * *
 CAG TCC GGA GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA
 Gln Ser Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu>

1450 1460 1470 1480
 * * * * *
 CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC
 Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp>

1490 1500 1510 1520 1530
 * * * * *
 ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC
 Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp>

1540 1550 1560 1570 1580
 * * * * *
 GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC
 Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly>

1590 1600 1610 1620 1630
 * * * * *
 GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC
 Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn>

1640 1650 1660 1670 1680
 * * * * *
 AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG
 Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp>

1690 1700 1710 1720
 * * * * *
 CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA
 Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro>

1730 1740 1750 1760 1770
 * * * * *
 GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA
 Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu>

Figure 21D

```

1780      1790      1800      1810      1820
*      *      *      *      *
CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAG GAG ATG ACC AAG AAC
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn>

      1830      1840      1850      1860      1870
*      *      *      *      *
CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile>

      1880      1890      1900      1910      1920
*      *      *      *      *
GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr>

      1930      1940      1950      1960
*      *      *      *
ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAT AGC AAG
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys>

1970      1980      1990      2000      2010
*      *      *      *      *
CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys>

      2020      2030      2040      2050      2060
*      *      *      *      *
TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu>

      2070      2080
*      *      *
TCC CTG TCT CCG GGT AAA TGA
Ser Leu Ser Pro Gly Lys ***>

```

Figure 22A

```

      10      20      30      40
      *      *      *      *
ATG GTG AAG CCA TCA TTA CCA TTC ACA TCC CTC TTA TTC CTG CAG CTG
Met Val Lys Pro Ser Leu Pro Phe Thr Ser Leu Leu Phe Leu Gln Leu>

50      60      70      80      90
      *      *      *      *      *
CCC CTG CTG GGA GTG GGG CTG AAC ACG ACA ATT CTG ACG CCC AAT GGG
Pro Leu Leu Gly Val Gly Leu Asn Thr Thr Ile Leu Thr Pro Asn Gly>

100      110      120      130      140
      *      *      *      *      *
AAT GAA GAC ACC ACA GCT GAT TTC TTC CTG ACC ACT ATG CCC ACT GAC
Asn Glu Asp Thr Thr Ala Asp Phe Phe Leu Thr Thr Met Pro Thr Asp>

150      160      170      180      190
      *      *      *      *      *
TCC CTC AGT GTT TCC ACT CTG CCC CTC CCA GAG GTT CAG TGT TTT GTG
Ser Leu Ser Val Ser Thr Leu Pro Leu Pro Glu Val Gln Cys Phe Val>

200      210      220      230      240
      *      *      *      *      *
TTC AAT GTC GAG TAC ATG AAT TGC ACT TGG AAC AGC AGC TCT GAG CCC
Phe Asn Val Glu Tyr Met Asn Cys Thr Trp Asn Ser Ser Ser Glu Pro>

250      260      270      280
      *      *      *      *
CAG CCT ACC AAC CTC ACT CTG CAT TAT TGG TAC AAG AAC TCG GAT AAT
Gln Pro Thr Asn Leu Thr Leu His Tyr Trp Tyr Lys Asn Ser Asp Asn>

290      300      310      320      330
      *      *      *      *      *
GAT AAA GTC CAG AAG TGC AGC CAC TAT CTA TTC TCT GAA GAA ATC ACT
Asp Lys Val Gln Lys Cys Ser His Tyr Leu Phe Ser Glu Glu Ile Thr>

340      350      360      370      380
      *      *      *      *      *
TCT GGC TGT CAG TTG CAA AAA AAG GAG ATC CAC CTC TAC CAA ACA TTT
Ser Gly Cys Gln Leu Gln Lys Lys Glu Ile His Leu Tyr Gln Thr Phe>

390      400      410      420      430
      *      *      *      *      *
GTT GTT CAG CTC CAG GAC CCA CGG GAA CCC AGG AGA CAG GCC ACA CAG
Val Val Gln Leu Gln Asp Pro Arg Glu Pro Arg Arg Gln Ala Thr Gln>

440      450      460      470      480
      *      *      *      *      *
ATG CTA AAA CTG CAG AAT CTG GTG ATC CCC TGG GCT CCA GAG AAC CTA
Met Leu Lys Leu Gln Asn Leu Val Ile Pro Trp Ala Pro Glu Asn Leu>

490      500      510      520
      *      *      *      *
ACA CTT CAC AAA CTG AGT GAA TCC CAG CTA GAA CTG AAC TGG AAC AAC
Thr Leu His Lys Leu Ser Glu Ser Gln Leu Glu Leu Asn Trp Asn Asn>

530      540      550      560      570
      *      *      *      *      *
AGA TTC TTG AAC CAC TGT TTG GAG CAC TTG GTG CAG TAC CGG ACT GAC
Arg Phe Leu Asn His Cys Leu Glu His Leu Val Gln Tyr Arg Thr Asp>

```

```

580      590      600      610      620
*      *      *      *      *
TGG GAC CAC AGC TGG ACT GAA CAA TCA GTG GAT TAT AGA CAT AAG TTC
Trp Asp His Ser Trp Thr Glu Gln Ser Val Asp Tyr Arg His Lys Phe>

630      640      650      660      670
*      *      *      *      *
TCC TTG CCT AGT GTG GAT GGG CAG AAA CGC TAC ACG TTT CGT GTT CGG
Ser Leu Pro Ser Val Asp Gly Gln Lys Arg Tyr Thr Phe Arg Val Arg>

680      690      700      710      720
*      *      *      *      *
AGC CGC TTT AAC CCA CTC TGT GGA AGT GCT CAG CAT TGG AGT GAA TGG
Ser Arg Phe Asn Pro Leu Cys Gly Ser Ala Gln His Trp Ser Glu Trp>

730      740      750      760
*      *      *      *      *
AGC CAC CCA ATC CAC TGG GGG AGC AAT ACT TCA AAA GAG AAC GGG AAC
Ser His Pro Ile His Trp Gly Ser Asn Thr Ser Lys Glu Asn Gly Asn>

770      780      790      800      810
*      *      *      *      *
ATG AAG GTC CTG CAG GAG CCC ACC TGC GTC TCC GAC TAC ATG AGC ATC
Met Lys Val Leu Gln Glu Pro Thr Cys Val Ser Asp Tyr Met Ser Ile>

820      830      840      850      860
*      *      *      *      *
TCT ACT TGC GAG TGG AAG ATG AAT GGT CCC ACC AAT TGC AGC ACC GAG
Ser Thr Cys Glu Trp Lys Met Asn Gly Pro Thr Asn Cys Ser Thr Glu>

870      880      890      900      910
*      *      *      *      *
CTC CGC CTG TTG TAC CAG CTG GTT TTT CTG CTC TCC GAA GCC CAC ACG
Leu Arg Leu Leu Tyr Gln Leu Val Phe Leu Leu Ser Glu Ala His Thr>

920      930      940      950      960
*      *      *      *      *
TGT ATC CCT GAG AAC AAC GGA GGC GCG GGG TGC GTG TGC CAC CTG CTC
Cys Ile Pro Glu Asn Asn Gly Gly Ala Gly Cys Val Cys His Leu Leu>

970      980      990      1000
*      *      *      *      *
ATG GAT GAC GTG GTC AGT GCG GAT AAC TAT ACA CTG GAC CTG TGG GCT
Met Asp Asp Val Val Ser Ala Asp Asn Tyr Thr Leu Asp Leu Trp Ala>

1010      1020      1030      1040      1050
*      *      *      *      *
GGG CAG CAG CTG CTG TGG AAG GGC TCC TTC AAG CCC AGC GAG CAT GTG
Gly Gln Gln Leu Leu Trp Lys Gly Ser Phe Lys Pro Ser Glu His Val>

1060      1070      1080      1090      1100
*      *      *      *      *
AAA CCC AGG GCC CCA GGA AAC CTG ACA GTT CAC ACC AAT GTC TCC GAC
Lys Pro Arg Ala Pro Gly Asn Leu Thr Val His Thr Asn Val Ser Asp>

1110      1120      1130      1140      1150
*      *      *      *      *
ACT CTG CTG CTG ACC TGG AGC AAC CCG TAT CCC CCT GAC AAT TAC CTG
Thr Leu Leu Leu Thr Trp Ser Asn Pro Tyr Pro Pro Asp Asn Tyr Leu>

1160      1170      1180      1190      1200
*      *      *      *      *

```

TAT AAT CAT CTC ACC TAT GCA GTC AAC ATT TGG AGT GAA AAC GAC CCG
 Tyr Asn His Leu Thr Tyr Ala Val Asn Ile Trp Ser Glu Asn Asp Pro>

1210 1220 1230 1240
 * * * * *
 GCA GAT TTC AGA ATC TAT AAC GTG ACC TAC CTA GAA CCC TCC CTC CGC
 Ala Asp Phe Arg Ile Tyr Asn Val Thr Tyr Leu Glu Pro Ser Leu Arg>

1250 1260 1270 1280 1290
 * * * * *
 ATC GCA GCC AGC ACC CTG AAG TCT GGG ATT TCC TAC AGG GCA CGG GTG
 Ile Ala Ala Ser Thr Leu Lys Ser Gly Ile Ser Tyr Arg Ala Arg Val>

1300 1310 1320 1330 1340
 * * * * *
 AGG GCC TGG GCT CAG AGC TAT AAC ACC ACC TGG AGT GAG TGG AGC CCC
 Arg Ala Trp Ala Gln Ser Tyr Asn Thr Thr Trp Ser Glu Trp Ser Pro>

1350 1360 1370 1380 1390
 * * * * *
 AGC ACC AAG TGG CAC AAC TCC TAC AGG GAG CCC TTC GAG CAG TCC GGA
 Ser Thr Lys Trp His Asn Ser Tyr Arg Glu Pro Phe Glu Gln Ser Gly>

1400 1410 1420 1430 1440
 * * * * *
 GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG
 Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly>

1450 1460 1470 1480
 * * * * *
 GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG
 Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met>

1490 1500 1510 1520 1530
 * * * * *
 ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC
 Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His>

1540 1550 1560 1570 1580
 * * * * *
 GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG
 Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val>

1590 1600 1610 1620 1630
 * * * * *
 CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC
 His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr>

1640 1650 1660 1670 1680
 * * * * *
 CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC
 Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly>

1690 1700 1710 1720
 * * * * *
 AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC
 Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile>

1730 1740 1750 1760 1770
 * * * * *
 GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG
 Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val>

```

1780      1790      1800      1810      1820
*          *          *          *          *
TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser>

      1830      1840      1850      1860      1870
*          *          *          *          *
CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu>

      1880      1890      1900      1910      1920
*          *          *          *          *
TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro>

      1930      1940      1950      1960
*          *          *          *          *
GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAT AGC AAG CTC ACC GTG
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val>

1970      1980      1990      2000      2010
*          *          *          *          *
GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met>

      2020      2030      2040      2050      2060
*          *          *          *          *
CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser>

      2070
*          *          *
CCG GGT AAA TGA
Pro Gly Lys ***>

```


Figure 23A

```

      10      20      30      40
      *      *      *      *
ATG GTG AAG CCA TCA TTA CCA TTC ACA TCC CTC TTA TTC CTG CAG CTG
Met Val Lys Pro Ser Leu Pro Phe Thr Ser Leu Leu Phe Leu Gln Leu>

50      60      70      80      90
      *      *      *      *      *
CCC CTG CTG GGA GTG GGG CTG AAC ACG ACA ATT CTG ACG CCC AAT GGG
Pro Leu Leu Gly Val Gly Leu Asn Thr Thr Ile Leu Thr Pro Asn Gly>

100     110     120     130     140
      *      *      *      *      *
AAT GAA GAC ACC ACA GCT GAT TTC TTC CTG ACC ACT ATG CCC ACT GAC
Asn Glu Asp Thr Thr Ala Asp Phe Phe Leu Thr Thr Met Pro Thr Asp>

150     160     170     180     190
      *      *      *      *      *
TCC CTC AGT GTT TCC ACT CTG CCC CTC CCA GAG GTT CAG TGT TTT GTG
Ser Leu Ser Val Ser Thr Leu Pro Leu Pro Glu Val Gln Cys Phe Val>

200     210     220     230     240
      *      *      *      *      *
TTC AAT GTC GAG TAC ATG AAT TGC ACT TGG AAC AGC AGC TCT GAG CCC
Phe Asn Val Glu Tyr Met Asn Cys Thr Trp Asn Ser Ser Ser Glu Pro>

250     260     270     280
      *      *      *      *
CAG CCT ACC AAC CTC ACT CTG CAT TAT TGG TAC AAG AAC TCG GAT AAT
Gln Pro Thr Asn Leu Thr Leu His Tyr Trp Tyr Lys Asn Ser Asp Asn>

290     300     310     320     330
      *      *      *      *      *
GAT AAA GTC CAG AAG TGC AGC CAC TAT CTA TTC TCT GAA GAA ATC ACT
Asp Lys Val Gln Lys Cys Ser His Tyr Leu Phe Ser Glu Glu Ile Thr>

340     350     360     370     380
      *      *      *      *      *
TCT GGC TGT CAG TTG CAA AAA AAG GAG ATC CAC CTC TAC CAA ACA TTT
Ser Gly Cys Gln Leu Gln Lys Lys Glu Ile His Leu Tyr Gln Thr Phe>

390     400     410     420     430
      *      *      *      *      *
GTT GTT CAG CTC CAG GAC CCA CGG GAA CCC AGG AGA CAG GCC ACA CAG
Val Val Gln Leu Gln Asp Pro Arg Glu Pro Arg Arg Gln Ala Thr Gln>

440     450     460     470     480
      *      *      *      *      *
ATG CTA AAA CTG CAG AAT CTG GTG ATC CCC TGG GCT CCA GAG AAC CTA
Met Leu Lys Leu Gln Asn Leu Val Ile Pro Trp Ala Pro Glu Asn Leu>

490     500     510     520
      *      *      *      *
ACA CTT CAC AAA CTG AGT GAA TCC CAG CTA GAA CTG AAC TGG AAC AAC
Thr Leu His Lys Leu Ser Glu Ser Gln Leu Glu Leu Asn Trp Asn Asn>

530     540     550     560     570
      *      *      *      *      *
AGA TTC TTG AAC CAC TGT TTG GAG CAC TTG GTG CAG TAC CGG ACT GAC
Arg Phe Leu Asn His Cys Leu Glu His Leu Val Gln Tyr Arg Thr Asp>

```

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Figure 23B

```

580      590      600      610      620
*      *      *      *      *
TGG GAC CAC AGC TGG ACT GAA CAA TCA GTG GAT TAT AGA CAT AAG TTC
Trp Asp His Ser Trp Thr Glu Gln Ser Val Asp Tyr Arg His Lys Phe>

630      640      650      660      670
*      *      *      *      *
TCC TTG CCT AGT GTG GAT GGG CAG AAA CGC TAC ACG TTT CGT GTT CGG
Ser Leu Pro Ser Val Asp Gly Gln Lys Arg Tyr Thr Phe Arg Val Arg>

680      690      700      710      720
*      *      *      *      *
AGC CGC TTT AAC CCA CTC TGT GGA AGT GCT CAG CAT TGG AGT GAA TGG
Ser Arg Phe Asn Pro Leu Cys Gly Ser Ala Gln His Trp Ser Glu Trp>

730      740      750      760
*      *      *      *
AGC CAC CCA ATC CAC TGG GGG AGC AAT ACT TCA AAA GAG AAC GCG TCG
Ser His Pro Ile His Trp Gly Ser Asn Thr Ser Lys Glu Asn Ala Ser>

770      780      790      800      810
*      *      *      *      *
TCT GGG AAC ATG AAG GTC CTG CAG GAG CCC ACC TGC GTC TCC GAC TAC
Ser Gly Asn Met Lys Val Leu Gln Glu Pro Thr Cys Val Ser Asp Tyr>

820      830      840      850      860
*      *      *      *      *
ATG AGC ATC TCT ACT TGC GAG TGG AAG ATG AAT GGT CCC ACC AAT TGC
Met Ser Ile Ser Thr Cys Glu Trp Lys Met Asn Gly Pro Thr Asn Cys>

870      880      890      900      910
*      *      *      *      *
AGC ACC GAG CTC CGC CTG TTG TAC CAG CTG GTT TTT CTG CTC TCC GAA
Ser Thr Glu Leu Arg Leu Leu Tyr Gln Leu Val Phe Leu Leu Ser Glu>

920      930      940      950      960
*      *      *      *      *
GCC CAC ACG TGT ATC CCT GAG AAC AAC GGA GGC GCG GGG TGC GTG TGC
Ala His Thr Cys Ile Pro Glu Asn Asn Gly Gly Ala Gly Cys Val Cys>

970      980      990      1000
*      *      *      *
CAC CTG CTC ATG GAT GAC GTG GTC AGT GCG GAT AAC TAT ACA CTG GAC
His Leu Leu Met Asp Asp Val Val Ser Ala Asp Asn Tyr Thr Leu Asp>

1010      1020      1030      1040      1050
*      *      *      *      *
CTG TGG GCT GGG CAG CAG CTG CTG TGG AAG GGC TCC TTC AAG CCC AGC
Leu Trp Ala Gly Gln Gln Leu Leu Trp Lys Gly Ser Phe Lys Pro Ser>

1060      1070      1080      1090      1100
*      *      *      *      *
GAG CAT GTG AAA CCC AGG GCC CCA GGA AAC CTG ACA GTT CAC ACC AAT
Glu His Val Lys Pro Arg Ala Pro Gly Asn Leu Thr Val His Thr Asn>

1110      1120      1130      1140      1150
*      *      *      *      *
GTC TCC GAC ACT CTG CTG CTG ACC TGG AGC AAC CCG TAT CCC CCT GAC
Val Ser Asp Thr Leu Leu Leu Thr Trp Ser Asn Pro Tyr Pro Pro Asp>

1160      1170      1180      1190      1200
*      *      *      *      *

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AAT TAC CTG TAT AAT CAT CTC ACC TAT GCA GTC AAC ATT TGG AGT GAA
 Asn Tyr Leu Tyr Asn His Leu Thr Tyr Ala Val Asn Ile Trp Ser Glu>

1210 1220 1230 1240
 * * * * *
 AAC GAC CCG GCA GAT TTC AGA ATC TAT AAC GTG ACC TAC CTA GAA CCC
 Asn Asp Pro Ala Asp Phe Arg Ile Tyr Asn Val Thr Tyr Leu Glu Pro>

1250 1260 1270 1280 1290
 * * * * *
 TCC CTC CGC ATC GCA GCC AGC ACC CTG AAG TCT GGG ATT TCC TAC AGG
 Ser Leu Arg Ile Ala Ala Ser Thr Leu Lys Ser Gly Ile Ser Tyr Arg>

1300 1310 1320 1330 1340
 * * * * *
 GCA CGG GTG AGG GCC TGG GCT CAG AGC TAT AAC ACC ACC TGG AGT GAG
 Ala Arg Val Arg Ala Trp Ala Gln Ser Tyr Asn Thr Thr Trp Ser Glu>

1350 1360 1370 1380 1390
 * * * * *
 TGG AGC CCC AGC ACC AAG TGG CAC AAC TCC TAC AGG GAG CCC TTC GAG
 Trp Ser Pro Ser Thr Lys Trp His Asn Ser Tyr Arg Glu Pro Phe Glu>

1400 1410 1420 1430 1440
 * * * * *
 CAG TCC GGA GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA
 Gln Ser Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu>

1450 1460 1470 1480
 * * * * *
 CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC
 Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp>

1490 1500 1510 1520 1530
 * * * * *
 ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC
 Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp>

1540 1550 1560 1570 1580
 * * * * *
 GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC
 Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly>

1590 1600 1610 1620 1630
 * * * * *
 GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC
 Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn>

1640 1650 1660 1670 1680
 * * * * *
 AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG
 Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp>

1690 1700 1710 1720
 * * * * *
 CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA
 Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro>

1730 1740 1750 1760 1770
 * * * * *
 GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA
 Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu>

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1780      1790      1800      1810      1820
*          *          *          *          *
CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn>

      1830      1840      1850      1860      1870
*          *          *          *          *
CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile>

      1880      1890      1900      1910      1920
*          *          *          *          *
GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr>

      1930      1940      1950      1960
*          *          *          *          *
ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAT AGC AAG
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys>

1970      1980      1990      2000      2010
*          *          *          *          *
CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys>

      2020      2030      2040      2050      2060
*          *          *          *          *
TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu>

      2070      2080
*          *          *          *          *
TCC CTG TCT CCG GGT AAA TGA
Ser Leu Ser Pro Gly Lys ***>

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      10      20      30      40
      *      *      *      *
ATG GTG GCC GTC GGC TGC GCG CTG CTG GCT GCC CTG CTG GCC GCG CCG
Met Val Ala Val Gly Cys Ala Leu Leu Ala Ala Leu Ala Ala Pro>

50      60      70      80      90
      *      *      *      *      *
GGA GCG GCG CTG GCC CCA AGG CGC TGC CCT GCG CAG GAG GTG GCA AGA
Gly Ala Ala Leu Ala Pro Arg Arg Cys Pro Ala Gln Glu Val Ala Arg>

100     110     120     130     140
      *      *      *      *      *
GGC GTG CTG ACC AGT CTG CCA GGA GAC AGC GTG ACT CTG ACC TGC CCG
Gly Val Leu Thr Ser Leu Pro Gly Asp Ser Val Thr Leu Thr Cys Pro>

150     160     170     180     190
      *      *      *      *      *
GGG GTA GAG CCG GAA GAC AAT GCC ACT GTT CAC TGG GTG CTC AGG AAG
Gly Val Glu Pro Glu Asp Asn Ala Thr Val His Trp Val Leu Arg Lys>

200     210     220     230     240
      *      *      *      *      *
CCG GCT GCA GGC TCC CAC CCC AGC AGA TGG GCT GGC ATG GGA AGG AGG
Pro Ala Ala Gly Ser His Pro Ser Arg Trp Ala Gly Met Gly Arg Arg>

250     260     270     280
      *      *      *      *
CTG CTG CTG AGG TCG GTG CAG CTC CAC GAC TCT GGA AAC TAT TCA TGC
Leu Leu Leu Arg Ser Val Gln Leu His Asp Ser Gly Asn Tyr Ser Cys>

290     300     310     320     330
      *      *      *      *      *
TAC CGG GCC GGC CGC CCA GCT GGG ACT GTG CAC TTG CTG GTG GAT GTT
Tyr Arg Ala Gly Arg Pro Ala Gly Thr Val His Leu Leu Val Asp Val>

340     350     360     370     380
      *      *      *      *      *
CCC CCC GAG GAG CCC CAG CTC TCC TGC TTC CGG AAG AGC CCC CTC AGC
Pro Pro Glu Glu Pro Gln Leu Ser Cys Phe Arg Lys Ser Pro Leu Ser>

390     400     410     420     430
      *      *      *      *      *
AAT GTT GTT TGT GAG TGG GGT CCT CGG AGC ACC CCA TCC CTG ACG ACA
Asn Val Val Cys Glu Trp Gly Pro Arg Ser Thr Pro Ser Leu Thr Thr>

440     450     460     470     480
      *      *      *      *      *
AAG GCT GTG CTC TTG GTG AGG AAG TTT CAG AAC AGT CCG GCC GAA GAC
Lys Ala Val Leu Leu Val Arg Lys Phe Gln Asn Ser Pro Ala Glu Asp>

490     500     510     520
      *      *      *      *
TTC CAG GAG CCG TGC CAG TAT TCC CAG GAG TCC CAG AAG TTC TCC TGC
Phe Gln Glu Pro Cys Gln Tyr Ser Gln Glu Ser Gln Lys Phe Ser Cys>

530     540     550     560     570
      *      *      *      *      *
CAG TTA GCA GTC CCG GAG GGA GAC AGC TCT TTC TAC ATA GTG TCC ATG
Gln Leu Ala Val Pro Glu Gly Asp Ser Ser Phe Tyr Ile Val Ser Met>

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Figure 24B

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      580      590      600      610      620
      *      *      *      *      *
TGC GTC GCC AGT AGT GTC GGG AGC AAG TTC AGC AAA ACT CAA ACC TTT
Cys Val Ala Ser Ser Val Gly Ser Lys Phe Ser Lys Thr Gln Thr Phe>

      630      640      650      660      670
      *      *      *      *      *
CAG GGT TGT GGA ATC TTG CAG CCT GAT CCG CCT GCC AAC ATC ACA GTC
Gln Gly Cys Gly Ile Leu Gln Pro Asp Pro Pro Ala Asn Ile Thr Val>

      680      690      700      710      720
      *      *      *      *      *
ACT GCC GTG GCC AGA AAC CCC CGC TGG CTC AGT GTC ACC TGG CAA GAC
Thr Ala Val Ala Arg Asn Pro Arg Trp Leu Ser Val Thr Trp Gln Asp>

      730      740      750      760
      *      *      *      *
CCC CAC TCC TGG AAC TCA TCT TTC TAC AGA CTA CGG TTT GAG CTC AGA
Pro His Ser Trp Asn Ser Ser Phe Tyr Arg Leu Arg Phe Glu Leu Arg>

      770      780      790      800      810
      *      *      *      *      *
TAT CGG GCT GAA CGG TCA AAG ACA TTC ACA ACA TGG ATG GTC AAG GAC
Tyr Arg Ala Glu Arg Ser Lys Thr Phe Thr Thr Trp Met Val Lys Asp>

      820      830      840      850      860
      *      *      *      *      *
CTC CAG CAT CAC TGT GTC ATC CAC GAC GCC TGG AGC GGC CTG AGG CAC
Leu Gln His His Cys Val Ile His Asp Ala Trp Ser Gly Leu Arg His>

      870      880      890      900      910
      *      *      *      *      *
GTG GTG CAG CTT CGT GCC CAG GAG GAG TTC GGG CAA GGC GAG TGG AGC
Val Val Gln Leu Arg Ala Gln Glu Glu Phe Gly Gln Gly Glu Trp Ser>

      920      930      940      950      960
      *      *      *      *      *
GAG TGG AGC CCG GAG GCC ATG GGC ACG CCT TGG ACA GAA TCC AGG AGT
Glu Trp Ser Pro Glu Ala Met Gly Thr Pro Trp Thr Glu Ser Arg Ser>

      970      980      990      1000
      *      *      *      *
CCT CCA GCT GAG AAC GAG GTG TCC ACC CCC ATG ACC GGT GGC GCG CCT
Pro Pro Ala Glu Asn Glu Val Ser Thr Pro Met Thr Gly Gly Ala Pro>

      1010      1020      1030      1040      1050
      *      *      *      *      *
TCA GGT GCT CAG CTG GAA CTT CTA GAC CCA TGT GGT TAT ATC AGT CCT
Ser Gly Ala Gln Leu Glu Leu Leu Asp Pro Cys Gly Tyr Ile Ser Pro>

      1060      1070      1080      1090      1100
      *      *      *      *      *
GAA TCT CCA GTT GTA CAA CTT CAT TCT AAT TTC ACT GCA GTT TGT GTG
Glu Ser Pro Val Val Gln Leu His Ser Asn Phe Thr Ala Val Cys Val>

      1110      1120      1130      1140      1150
      *      *      *      *      *
CTA AAG GAA AAA TGT ATG GAT TAT TTT CAT GTA AAT GCT AAT TAC ATT
Leu Lys Glu Lys Cys Met Asp Tyr Phe His Val Asn Ala Asn Tyr Ile>

      1160      1170      1180      1190      1200
      *      *      *      *      *

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Figure 24C

GTC TGG AAA ACA AAC CAT TTT ACT ATT CCT AAG GAG CAA TAT ACT ATC
Val Trp Lys Thr Asn His Phe Thr Ile Pro Lys Glu Gln Tyr Thr Ile>

1210 1220 1230 1240
* * * * *
ATA AAC AGA ACA GCA TCC AGT GTC ACC TTT ACA GAT ATA GCT TCA TTA
Ile Asn Arg Thr Ala Ser Ser Val Thr Phe Thr Asp Ile Ala Ser Leu>

1250 1260 1270 1280 1290
* * * * *
AAT ATT CAG CTC ACT TGC AAC ATT CTT ACA TTC GGA CAG CTT GAA CAG
Asn Ile Gln Leu Thr Cys Asn Ile Leu Thr Phe Gly Gln Leu Glu Gln>

1300 1310 1320 1330 1340
* * * * *
AAT GTT TAT GGA ATC ACA ATA ATT TCA GGC TTG CCT CCA GAA AAA CCT
Asn Val Tyr Gly Ile Thr Ile Ile Ser Gly Leu Pro Pro Glu Lys Pro>

1350 1360 1370 1380 1390
* * * * *
AAA AAT TTG AGT TGC ATT GTG AAC GAG GGG AAG AAA ATG AGG TGT GAG
Lys Asn Leu Ser Cys Ile Val Asn Glu Gly Lys Lys Met Arg Cys Glu>

1400 1410 1420 1430 1440
* * * * *
TGG GAT GGT GGA AGG GAA ACA CAC TTG GAG ACA AAC TTC ACT TTA AAA
Trp Asp Gly Gly Arg Glu Thr His Leu Glu Thr Asn Phe Thr Leu Lys>

1450 1460 1470 1480
* * * * *
TCT GAA TGG GCA ACA CAC AAG TTT GCT GAT TGC AAA GCA AAA CGT GAC
Ser Glu Trp Ala Thr His Lys Phe Ala Asp Cys Lys Ala Lys Arg Asp>

1490 1500 1510 1520 1530
* * * * *
ACC CCC ACC TCA TGC ACT GTT GAT TAT TCT ACT GTG TAT TTT GTC AAC
Thr Pro Thr Ser Cys Thr Val Asp Tyr Ser Thr Val Tyr Phe Val Asn>

1540 1550 1560 1570 1580
* * * * *
ATT GAA GTC TGG GTA GAA GCA GAG AAT GCC CTT GGG AAG GTT ACA TCA
Ile Glu Val Trp Val Glu Ala Glu Asn Ala Leu Gly Lys Val Thr Ser>

1590 1600 1610 1620 1630
* * * * *
GAT CAT ATC AAT TTT GAT CCT GTA TAT AAA GTG AAG CCC AAT CCG CCA
Asp His Ile Asn Phe Asp Pro Val Tyr Lys Val Lys Pro Asn Pro Pro>

1640 1650 1660 1670 1680
* * * * *
CAT AAT TTA TCA GTG ATC AAC TCA GAG GAA CTG TCT AGT ATC TTA AAA
His Asn Leu Ser Val Ile Asn Ser Glu Glu Leu Ser Ser Ile Leu Lys>

1690 1700 1710 1720
* * * * *
TTG ACA TGG ACC AAC CCA AGT ATT AAG AGT GTT ATA ATA CTA AAA TAT
Leu Thr Trp Thr Asn Pro Ser Ile Lys Ser Val Ile Ile Leu Lys Tyr>

1730 1740 1750 1760 1770
* * * * *
AAC ATT CAA TAT AGG ACC AAA GAT GCC TCA ACT TGG AGC CAG ATT CCT
Asn Ile Gln Tyr Arg Thr Lys Asp Ala Ser Thr Trp Ser Gln Ile Pro>

Figure 24D

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1780      1790      1800      1810      1820
*          *          *          *          *
CCT GAA GAC ACA GCA TCC ACC CGA TCT TCA TTC ACT GTC CAA GAC CTT
Pro Glu Asp Thr Ala Ser Thr Arg Ser Ser Phe Thr Val Gln Asp Leu>

1830      1840      1850      1860      1870
*          *          *          *          *
AAA CCT TTT ACA GAA TAT GTG TTT AGG ATT CGC TGT ATG AAG GAA GAT
Lys Pro Phe Thr Glu Tyr Val Phe Arg Ile Arg Cys Met Lys Glu Asp>

1880      1890      1900      1910      1920
*          *          *          *          *
GGT AAG GGA TAC TGG AGT GAC TGG AGT GAA GAA GCA AGT GGG ATC ACC
Gly Lys Gly Tyr Trp Ser Asp Trp Ser Glu Glu Ala Ser Gly Ile Thr>

1930      1940      1950      1960
*          *          *          *          *
TAT GAA GAT AGA CCA TCT AAA GCA CCA AGT TTC TGG TAT AAA ATA GAT
Tyr Glu Asp Arg Pro Ser Lys Ala Pro Ser Phe Trp Tyr Lys Ile Asp>

1970      1980      1990      2000      2010
*          *          *          *          *
CCA TCC CAT ACT CAA GGC TAC AGA ACT GTA CAA CTC GTG TGG AAG ACA
Pro Ser His Thr Gln Gly Tyr Arg Thr Val Gln Leu Val Trp Lys Thr>

2020      2030      2040      2050      2060
*          *          *          *          *
TTG CCT CCT TTT GAA GCC AAT GGA AAA ATC TTG GAT TAT GAA GTG ACT
Leu Pro Pro Phe Glu Ala Asn Gly Lys Ile Leu Asp Tyr Glu Val Thr>

2070      2080      2090      2100      2110
*          *          *          *          *
CTC ACA AGA TGG AAA TCA CAT TTA CAA AAT TAC ACA GTT AAT GCC ACA
Leu Thr Arg Trp Lys Ser His Leu Gln Asn Tyr Thr Val Asn Ala Thr>

2120      2130      2140      2150      2160
*          *          *          *          *
AAA CTG ACA GTA AAT CTC ACA AAT GAT CGC TAT CTA GCA ACC CTA ACA
Lys Leu Thr Val Asn Leu Thr Asn Asp Arg Tyr Leu Ala Thr Leu Thr>

2170      2180      2190      2200
*          *          *          *          *
GTA AGA AAT CTT GTT GGC AAA TCA GAT GCA GCT GTT TTA ACT ATC CCT
Val Arg Asn Leu Val Gly Lys Ser Asp Ala Ala Val Leu Thr Ile Pro>

2210      2220      2230      2240      2250
*          *          *          *          *
GCC TGT GAC TTT CAA GCT ACT CAC CCT GTA ATG GAT CTT AAA GCA TTC
Ala Cys Asp Phe Gln Ala Thr His Pro Val Met Asp Leu Lys Ala Phe>

2260      2270      2280      2290      2300
*          *          *          *          *
CCC AAA GAT AAC ATG CTT TGG GTG GAA TGG ACT ACT CCA AGG GAA TCT
Pro Lys Asp Asn Met Leu Trp Val Glu Trp Thr Thr Pro Arg Glu Ser>

2310      2320      2330      2340      2350
*          *          *          *          *
GTA AAG AAA TAT ATA CTT GAG TGG TGT GTG TTA TCA GAT AAA GCA CCC
Val Lys Lys Tyr Ile Leu Glu Trp Cys Val Leu Ser Asp Lys Ala Pro>

2360      2370      2380      2390      2400

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Figure 24E

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      *      *      *      *      *      *      *      *      *      *
TGT ATC ACA GAC TGG CAA CAA GAA GAT GGT ACC GTG CAT CGC ACC TAT
Cys Ile Thr Asp Trp Gln Gln Glu Asp Gly Thr Val His Arg Thr Tyr>

      2410      2420      2430      2440
      *      *      *      *      *      *      *      *
TTA AGA GGG AAC TTA GCA GAG AGC AAA TGC TAT TTG ATA ACA GTT ACT
Leu Arg Gly Asn Leu Ala Glu Ser Lys Cys Tyr Leu Ile Thr Val Thr>

2450      2460      2470      2480      2490
      *      *      *      *      *      *      *      *
CCA GTA TAT GCT GAT GGA CCA GGA AGC CCT GAA TCC ATA AAG GCA TAC
Pro Val Tyr Ala Asp Gly Pro Gly Ser Pro Glu Ser Ile Lys Ala Tyr>

      2500      2510      2520      2530      2540
      *      *      *      *      *      *      *      *
CTT AAA CAA GCT CCA CCT TCC AAA GGA CCT ACT GTT CGG ACA AAA AAA
Leu Lys Gln Ala Pro Pro Ser Lys Gly Pro Thr Val Arg Thr Lys Lys>

      2550      2560      2570      2580      2590
      *      *      *      *      *      *      *      *
GTA GGG AAA AAC GAA GCT GTC TTA GAG TGG GAC CAA CTT CCT GTT GAT
Val Gly Lys Asn Glu Ala Val Leu Glu Trp Asp Gln Leu Pro Val Asp>

      2600      2610      2620      2630      2640
      *      *      *      *      *      *      *      *
GTT CAG AAT GGA TTT ATC AGA AAT TAT ACT ATA TTT TAT AGA ACC ATC
Val Gln Asn Gly Phe Ile Arg Asn Tyr Thr Ile Phe Tyr Arg Thr Ile>

      2650      2660      2670      2680
      *      *      *      *      *      *      *      *
ATT GGA AAT GAA ACT GCT GTG AAT GTG GAT TCT TCC CAC ACA GAA TAT
Ile Gly Asn Glu Thr Ala Val Asn Val Asp Ser Ser His Thr Glu Tyr>

2690      2700      2710      2720      2730
      *      *      *      *      *      *      *      *
ACA TTG TCC TCT TTG ACT AGT GAC ACA TTG TAC ATG GTA CGA ATG GCA
Thr Leu Ser Ser Leu Thr Ser Asp Thr Leu Tyr Met Val Arg Met Ala>

      2740      2750      2760      2770      2780
      *      *      *      *      *      *      *      *
GCA TAC ACA GAT GAA GGT GGG AAG GAT GGT CCA GAA TTC ACT TTT ACT
Ala Tyr Thr Asp Glu Gly Gly Lys Asp Gly Pro Glu Phe Thr Phe Thr>

      2790      2800      2810      2820      2830
      *      *      *      *      *      *      *      *
ACC CCA AAG TTT GCT CAA GGA GAA ATT GAA TCC GGG GGC GAC AAA ACT
Thr Pro Lys Phe Ala Gln Gly Glu Ile Glu Ser Gly Gly Asp Lys Thr>

      2840      2850      2860      2870      2880
      *      *      *      *      *      *      *      *
CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser>

      2890      2900      2910      2920
      *      *      *      *      *      *      *      *
GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg>

2930      2940      2950      2960      2970
      *      *      *      *      *      *      *      *
ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT

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Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro>
2980      2990      3000      3010      3020
*          *          *          *          *
GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala>
3030      3040      3050      3060      3070
*          *          *          *          *
AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val>
3080      3090      3100      3110      3120
*          *          *          *          *
AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr>
3130      3140      3150      3160
*          *          *          *          *
AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr>
3170      3180      3190      3200      3210
*          *          *          *          *
ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu>
3220      3230      3240      3250      3260
*          *          *          *          *
CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC
Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys>
3270      3280      3290      3300      3310
*          *          *          *          *
CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser>
3320      3330      3340      3350      3360
*          *          *          *          *
AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp>
3370      3380      3390      3400
*          *          *          *          *
TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser>
3410      3420      3430      3440      3450
*          *          *          *          *
AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala>
3460      3470      3480      3490      3500
*          *          *          *          *
CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys>
*
TGA
***>

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Figure 25A

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      10      20      30      40
      *      *      *      *
ATG GTG GCC GTC GGC TGC GCG CTG CTG GCT GCC CTG CTG GCC GCG CCG
Met Val Ala Val Gly Cys Ala Leu Leu Ala Ala Leu Leu Ala Ala Pro>

      50      60      70      80      90
      *      *      *      *      *
GGA GCG GCG CTG GCC CCA AGG CGC TGC CCT GCG CAG GAG GTG GCA AGA
Gly Ala Ala Leu Ala Pro Arg Arg Cys Pro Ala Gln Glu Val Ala Arg>

     100     110     120     130     140
      *      *      *      *      *
GGC GTG CTG ACC AGT CTG CCA GGA GAC AGC GTG ACT CTG ACC TGC CCG
Gly Val Leu Thr Ser Leu Pro Gly Asp Ser Val Thr Leu Thr Cys Pro>

     150     160     170     180     190
      *      *      *      *      *
GGG GTA GAG CCG GAA GAC AAT GCC ACT GTT CAC TGG GTG CTC AGG AAG
Gly Val Glu Pro Glu Asp Asn Ala Thr Val His Trp Val Leu Arg Lys>

     200     210     220     230     240
      *      *      *      *      *
CCG GCT GCA GGC TCC CAC CCC AGC AGA TGG GCT GGC ATG GGA AGG AGG
Pro Ala Ala Gly Ser His Pro Ser Arg Trp Ala Gly Met Gly Arg Arg>

     250     260     270     280
      *      *      *      *
CTG CTG CTG AGG TCG GTG CAG CTC CAC GAC TCT GGA AAC TAT TCA TGC
Leu Leu Leu Arg Ser Val Gln Leu His Asp Ser Gly Asn Tyr Ser Cys>

    290     300     310     320     330
      *      *      *      *      *
TAC CGG GCC GGC CGC CCA GCT GGG ACT GTG CAC TTG CTG GTG GAT GTT
Tyr Arg Ala Gly Arg Pro Ala Gly Thr Val His Leu Leu Val Asp Val>

     340     350     360     370     380
      *      *      *      *      *
CCC CCC GAG GAG CCC CAG CTC TCC TGC TTC CGG AAG AGC CCC CTC AGC
Pro Pro Glu Glu Pro Gln Leu Ser Cys Phe Arg Lys Ser Pro Leu Ser>

     390     400     410     420     430
      *      *      *      *      *
AAT GTT GTT TGT GAG TGG GGT CCT CGG AGC ACC CCA TCC CTG ACG ACA
Asn Val Val Cys Glu Trp Gly Pro Arg Ser Thr Pro Ser Leu Thr Thr>

     440     450     460     470     480
      *      *      *      *      *
AAG GCT GTG CTC TTG GTG AGG AAG TTT CAG AAC AGT CCG GCC GAA GAC
Lys Ala Val Leu Leu Val Arg Lys Phe Gln Asn Ser Pro Ala Glu Asp>

     490     500     510     520
      *      *      *      *
TTC CAG GAG CCG TGC CAG TAT TCC CAG GAG TCC CAG AAG TTC TCC TGC
Phe Gln Glu Pro Cys Gln Tyr Ser Gln Glu Ser Gln Lys Phe Ser Cys>

    530     540     550     560     570
      *      *      *      *      *
CAG TTA GCA GTC CCG GAG GGA GAC AGC TCT TTC TAC ATA GTG TCC ATG
Gln Leu Ala Val Pro Glu Gly Asp Ser Ser Phe Tyr Ile Val Ser Met>

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Figure 25B

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580      590      600      610      620
*      *      *      *      *
TGC GTC GCC AGT AGT GTC GGG AGC AAG TTC AGC AAA ACT CAA ACC TTT
Cys Val Ala Ser Ser Val Gly Ser Lys Phe Ser Lys Thr Gln Thr Phe>

630      640      650      660      670
*      *      *      *      *
CAG GGT TGT GGA ATC TTG CAG CCT GAT CCG CCT GCC AAC ATC ACA GTC
Gln Gly Cys Gly Ile Leu Gln Pro Asp Pro Pro Ala Asn Ile Thr Val>

680      690      700      710      720
*      *      *      *      *
ACT GCC GTG GCC AGA AAC CCC CGC TGG CTC AGT GTC ACC TGG CAA GAC
Thr Ala Val Ala Arg Asn Pro Arg Trp Leu Ser Val Thr Trp Gln Asp>

730      740      750      760
*      *      *      *      *
CCC CAC TCC TGG AAC TCA TCT TTC TAC AGA CTA CGG TTT GAG CTC AGA
Pro His Ser Trp Asn Ser Ser Phe Tyr Arg Leu Arg Phe Glu Leu Arg>

770      780      790      800      810
*      *      *      *      *
TAT CGG GCT GAA CGG TCA AAG ACA TTC ACA ACA TGG ATG GTC AAG GAC
Tyr Arg Ala Glu Arg Ser Lys Thr Phe Thr Thr Trp Met Val Lys Asp>

820      830      840      850      860
*      *      *      *      *
CTC CAG CAT CAC TGT GTC ATC CAC GAC GCC TGG AGC GGC CTG AGG CAC
Leu Gln His His Cys Val Ile His Asp Ala Trp Ser Gly Leu Arg His>

870      880      890      900      910
*      *      *      *      *
GTG GTG CAG CTT CGT GCC CAG GAG GAG TTC GGG CAA GGC GAG TGG AGC
Val Val Gln Leu Arg Ala Gln Glu Glu Phe Gly Gln Gly Glu Trp Ser>

920      930      940      950      960
*      *      *      *      *
GAG TGG AGC CCG GAG GCC ATG GGC ACG CCT TGG ACA GAA TCG CGA TCG
Glu Trp Ser Pro Glu Ala Met Gly Thr Pro Trp Thr Glu Ser Arg Ser>

970      980      990      1000
*      *      *      *      *
CCT CCA GCT GAG AAC GAG GTG TCC ACC CCC ATG GAA CTT CTA GAC CCA
Pro Pro Ala Glu Asn Glu Val Ser Thr Pro Met Glu Leu Leu Asp Pro>

1010      1020      1030      1040      1050
*      *      *      *      *
TGT GGT TAT ATC AGT CCT GAA TCT CCA GTT GTA CAA CTT CAT TCT AAT
Cys Gly Tyr Ile Ser Pro Glu Ser Pro Val Val Gln Leu His Ser Asn>

1060      1070      1080      1090      1100
*      *      *      *      *
TTC ACT GCA GTT TGT GTG CTA AAG GAA AAA TGT ATG GAT TAT TTT CAT
Phe Thr Ala Val Cys Val Leu Lys Glu Lys Cys Met Asp Tyr Phe His>

1110      1120      1130      1140      1150
*      *      *      *      *
GTA AAT GCT AAT TAC ATT GTC TGG AAA ACA AAC CAT TTT ACT ATT CCT
Val Asn Ala Asn Tyr Ile Val Trp Lys Thr Asn His Phe Thr Ile Pro>

1160      1170      1180      1190      1200
*      *      *      *      *

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Figure 25C

AAG GAG CAA TAT ACT ATC ATA AAC AGA ACA GCA TCC AGT GTC ACC TTT
 Lys Glu Gln Tyr Thr Ile Ile Asn Arg Thr Ala Ser Ser Val Thr Phe>

1210 1220 1230 1240
 * * * * *
 ACA GAT ATA GCT TCA TTA AAT ATT CAG CTC ACT TGC AAC ATT CTT ACA
 Thr Asp Ile Ala Ser Leu Asn Ile Gln Leu Thr Cys Asn Ile Leu Thr>

1250 1260 1270 1280 1290
 * * * * *
 TTC GGA CAG CTT GAA CAG AAT GTT TAT GGA ATC ACA ATA ATT TCA GGC
 Phe Gly Gln Leu Glu Gln Asn Val Tyr Gly Ile Thr Ile Ile Ser Gly>

1300 1310 1320 1330 1340
 * * * * *
 TTG CCT CCA GAA AAA CCT AAA AAT TTG AGT TGC ATT GTG AAC GAG GGG
 Leu Pro Pro Glu Lys Pro Lys Asn Leu Ser Cys Ile Val Asn Glu Gly>

1350 1360 1370 1380 1390
 * * * * *
 AAG AAA ATG AGG TGT GAG TGG GAT GGT GGA AGG GAA ACA CAC TTG GAG
 Lys Lys Met Arg Cys Glu Trp Asp Gly Gly Arg Glu Thr His Leu Glu>

1400 1410 1420 1430 1440
 * * * * *
 ACA AAC TTC ACT TTA AAA TCT GAA TGG GCA ACA CAC AAG TTT GCT GAT
 Thr Asn Phe Thr Leu Lys Ser Glu Trp Ala Thr His Lys Phe Ala Asp>

1450 1460 1470 1480
 * * * * *
 TGC AAA GCA AAA CGT GAC ACC CCC ACC TCA TGC ACT GTT GAT TAT TCT
 Cys Lys Ala Lys Arg Asp Thr Pro Thr Ser Cys Thr Val Asp Tyr Ser>

1490 1500 1510 1520 1530
 * * * * *
 ACT GTG TAT TTT GTC AAC ATT GAA GTC TGG GTA GAA GCA GAG AAT GCC
 Thr Val Tyr Phe Val Asn Ile Glu Val Trp Val Glu Ala Glu Asn Ala>

1540 1550 1560 1570 1580
 * * * * *
 CTT GGG AAG GTT ACA TCA GAT CAT ATC AAT TTT GAT CCT GTA TAT AAA
 Leu Gly Lys Val Thr Ser Asp His Ile Asn Phe Asp Pro Val Tyr Lys>

1590 1600 1610 1620 1630
 * * * * *
 GTG AAG CCC AAT CCG CCA CAT AAT TTA TCA GTG ATC AAC TCA GAG GAA
 Val Lys Pro Asn Pro Pro His Asn Leu Ser Val Ile Asn Ser Glu Glu>

1640 1650 1660 1670 1680
 * * * * *
 CTG TCT AGT ATC TTA AAA TTG ACA TGG ACC AAC CCA AGT ATT AAG AGT
 Leu Ser Ser Ile Leu Lys Leu Thr Trp Thr Asn Pro Ser Ile Lys Ser>

1690 1700 1710 1720
 * * * * *
 GTT ATA ATA CTA AAA TAT AAC ATT CAA TAT AGG ACC AAA GAT GCC TCA
 Val Ile Ile Leu Lys Tyr Asn Ile Gln Tyr Arg Thr Lys Asp Ala Ser>

1730 1740 1750 1760 1770
 * * * * *
 ACT TGG AGC CAG ATT CCT CCT GAA GAC ACA GCA TCC ACC CGA TCT TCA
 Thr Trp Ser Gln Ile Pro Pro Glu Asp Thr Ala Ser Thr Arg Ser Ser>

Figure 25D

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1780      1790      1800      1810      1820
*          *          *          *          *
TTC ACT GTC CAA GAC CTT AAA CCT TTT ACA GAA TAT GTG TTT AGG ATT
Phe Thr Val Gln Asp Leu Lys Pro Phe Thr Glu Tyr Val Phe Arg Ile>

      1830      1840      1850      1860      1870
*          *          *          *          *
CGC TGT ATG AAG GAA GAT GGT AAG GGA TAC TGG AGT GAC TGG AGT GAA
Arg Cys Met Lys Glu Asp Gly Lys Gly Tyr Trp Ser Asp Trp Ser Glu>

      1880      1890      1900      1910      1920
*          *          *          *          *
GAA GCA AGT GGG ATC ACC TAT GAA GAT AGA CCA TCT AAA GCA CCA AGT
Glu Ala Ser Gly Ile Thr Tyr Glu Asp Arg Pro Ser Lys Ala Pro Ser>

      1930      1940      1950      1960
*          *          *          *          *
TTC TGG TAT AAA ATA GAT CCA TCC CAT ACT CAA GGC TAC AGA ACT GTA
Phe Trp Tyr Lys Ile Asp Pro Ser His Thr Gln Gly Tyr Arg Thr Val>

1970      1980      1990      2000      2010
*          *          *          *          *
CAA CTC GTG TGG AAG ACA TTG CCT CCT TTT GAA GCC AAT GGA AAA ATC
Gln Leu Val Trp Lys Thr Leu Pro Pro Phe Glu Ala Asn Gly Lys Ile>

      2020      2030      2040      2050      2060
*          *          *          *          *
TTG GAT TAT GAA GTG ACT CTC ACA AGA TGG AAA TCA CAT TTA CAA AAT
Leu Asp Tyr Glu Val Thr Leu Thr Arg Trp Lys Ser His Leu Gln Asn>

      2070      2080      2090      2100      2110
*          *          *          *          *
TAC ACA GTT AAT GCC ACA AAA CTG ACA GTA AAT CTC ACA AAT GAT CGC
Tyr Thr Val Asn Ala Thr Lys Leu Thr Val Asn Leu Thr Asn Asp Arg>

      2120      2130      2140      2150      2160
*          *          *          *          *
TAT CTA GCA ACC CTA ACA GTA AGA AAT CTT GTT GGC AAA TCA GAT GCA
Tyr Leu Ala Thr Leu Thr Val Arg Asn Leu Val Gly Lys Ser Asp Ala>

      2170      2180      2190      2200
*          *          *          *          *
GCT GTT TTA ACT ATC CCT GCC TGT GAC TTT CAA GCT ACT CAC CCT GTA
Ala Val Leu Thr Ile Pro Ala Cys Asp Phe Gln Ala Thr His Pro Val>

2210      2220      2230      2240      2250
*          *          *          *          *
ATG GAT CTT AAA GCA TTC CCC AAA GAT AAC ATG CTT TGG GTG GAA TGG
Met Asp Leu Lys Ala Phe Pro Lys Asp Asn Met Leu Trp Val Glu Trp>

      2260      2270      2280      2290      2300
*          *          *          *          *
ACT ACT CCA AGG GAA TCT GTA AAG AAA TAT ATA CTT GAG TGG TGT GTG
Thr Thr Pro Arg Glu Ser Val Lys Lys Tyr Ile Leu Glu Trp Cys Val>

      2310      2320      2330      2340      2350
*          *          *          *          *
TTA TCA GAT AAA GCA CCC TGT ATC ACA GAC TGG CAA CAA GAA GAT GGT
Leu Ser Asp Lys Ala Pro Cys Ile Thr Asp Trp Gln Gln Glu Asp Gly>

      2360      2370      2380      2390      2400

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Figure 25E

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*      *      *      *      *      *      *      *      *
ACC GTG CAT CGC ACC TAT TTA AGA GGG AAC TTA GCA GAG AGC AAA TGC
Thr Val His Arg Thr Tyr Leu Arg Gly Asn Leu Ala Glu Ser Lys Cys>

      2410      2420      2430      2440
*      *      *      *      *      *      *
TAT TTG ATA ACA GTT ACT CCA GTA TAT GCT GAT GGA CCA GGA AGC CCT
Tyr Leu Ile Thr Val Thr Pro Val Tyr Ala Asp Gly Pro Gly Ser Pro>

2450      2460      2470      2480      2490
*      *      *      *      *      *      *
GAA TCC ATA AAG GCA TAC CTT AAA CAA GCT CCA CCT TCC AAA GGA CCT
Glu Ser Ile Lys Ala Tyr Leu Lys Gln Ala Pro Pro Ser Lys Gly Pro>

      2500      2510      2520      2530      2540
*      *      *      *      *      *      *
ACT GTT CGG ACA AAA AAA GTA GGG AAA AAC GAA GCT GTC TTA GAG TGG
Thr Val Arg Thr Lys Lys Val Gly Lys Asn Glu Ala Val Leu Glu Trp>

      2550      2560      2570      2580      2590
*      *      *      *      *      *      *
GAC CAA CTT CCT GTT GAT GTT CAG AAT GGA TTT ATC AGA AAT TAT ACT
Asp Gln Leu Pro Val Asp Val Gln Asn Gly Phe Ile Arg Asn Tyr Thr>

      2600      2610      2620      2630      2640
*      *      *      *      *      *      *
ATA TTT TAT AGA ACC ATC ATT GGA AAT GAA ACT GCT GTG AAT GTG GAT
Ile Phe Tyr Arg Thr Ile Ile Gly Asn Glu Thr Ala Val Asn Val Asp>

      2650      2660      2670      2680
*      *      *      *      *      *
TCT TCC CAC ACA GAA TAT ACA TTG TCC TCT TTG ACT AGT GAC ACA TTG
Ser Ser His Thr Glu Tyr Thr Leu Ser Ser Leu Thr Ser Asp Thr Leu>

2690      2700      2710      2720      2730
*      *      *      *      *      *
TAC ATG GTA CGA ATG GCA GCA TAC ACA GAT GAA GGT GGG AAG GAT GGT
Tyr Met Val Arg Met Ala Ala Tyr Thr Asp Glu Gly Gly Lys Asp Gly>

      2740      2750      2760      2770      2780
*      *      *      *      *      *
CCA GAA TTC ACT TTT ACT ACC CCA AAG TTT GCT CAA GGA GAA ATT GAA
Pro Glu Phe Thr Phe Thr Thr Pro Lys Phe Ala Gln Gly Glu Ile Glu>

      2790      2800      2810      2820      2830
*      *      *      *      *      *
TCC GGG GGC GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA
Ser Gly Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu>

      2840      2850      2860      2870      2880
*      *      *      *      *      *
CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp>

      2890      2900      2910      2920
*      *      *      *      *
ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp>

2930      2940      2950      2960      2970
*      *      *      *      *
GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC

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Figure 25F

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly>

2980 2990 3000 3010 3020
 * * * * *
 GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC
 Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn>

3030 3040 3050 3060 3070
 * * * * *
 AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG
 Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp>

3080 3090 3100 3110 3120
 * * * * *
 CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA
 Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro>

3130 3140 3150 3160
 * * * *
 GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA
 Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu>

3170 3180 3190 3200 3210
 * * * * *
 CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC
 Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn>

3220 3230 3240 3250 3260
 * * * * *
 CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC
 Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile>

3270 3280 3290 3300 3310
 * * * * *
 GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC
 Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr>

3320 3330 3340 3350 3360
 * * * * *
 ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG
 Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys>

3370 3380 3390 3400
 * * * *
 CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC
 Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys>

3410 3420 3430 3440 3450
 * * * * *
 TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC
 Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu>

3460 3470
 * *
 TCC CTG TCT CCG GGT AAA TGA
 Ser Leu Ser Pro Gly Lys ****>

Figure 26A

```

      10      20      30      40
      *      *      *      *      *
ATG GTG CTT CTG TGG TGT GTA GTG AGT CTC TAC TTT TAT GGA ATC CTG
Met Val Leu Leu Trp Cys Val Val Ser Leu Tyr Phe Tyr Gly Ile Leu>

50      60      70      80      90
      *      *      *      *      *
CAA AGT GAT GCC TCA GAA CGC TGC GAT GAC TGG GGA CTA GAC ACC ATG
Gln Ser Asp Ala Ser Glu Arg Cys Asp Asp Trp Gly Leu Asp Thr Met>

100     110     120     130     140
      *      *      *      *      *
AGG CAA ATC CAA GTG TTT GAA GAT GAG CCA GCT CGC ATC AAG TGC CCA
Arg Gln Ile Gln Val Phe Glu Asp Glu Pro Ala Arg Ile Lys Cys Pro>

150     160     170     180     190
      *      *      *      *      *
CTC TTT GAA CAC TTC TTG AAA TTC AAC TAC AGC ACA GCC CAT TCA GCT
Leu Phe Glu His Phe Leu Lys Phe Asn Tyr Ser Thr Ala His Ser Ala>

200     210     220     230     240
      *      *      *      *      *
GGC CTT ACT CTG ATC TGG TAT TGG ACT AGG CAG GAC CGG GAC CTT GAG
Gly Leu Thr Leu Ile Trp Tyr Trp Thr Arg Gln Asp Arg Asp Leu Glu>

250     260     270     280
      *      *      *      *      *
GAG CCA ATT AAC TTC CGC CTC CCC GAG AAC CGC ATT AGT AAG GAG AAA
Glu Pro Ile Asn Phe Arg Leu Pro Glu Asn Arg Ile Ser Lys Glu Lys>

290     300     310     320     330
      *      *      *      *      *
GAT GTG CTG TGG TTC CGG CCC ACT CTC CTC AAT GAC ACT GGC AAC TAT
Asp Val Leu Trp Phe Arg Pro Thr Leu Leu Asn Asp Thr Gly Asn Tyr>

340     350     360     370     380
      *      *      *      *      *
ACC TGC ATG TTA AGG AAC ACT ACA TAT TGC AGC AAA GTT GCA TTT CCC
Thr Cys Met Leu Arg Asn Thr Thr Tyr Cys Ser Lys Val Ala Phe Pro>

390     400     410     420     430
      *      *      *      *      *
TTG GAA GTT GTT CAA AAA GAC AGC TGT TTC AAT TCC CCC ATG AAA CTC
Leu Glu Val Val Gln Lys Asp Ser Cys Phe Asn Ser Pro Met Lys Leu>

440     450     460     470     480
      *      *      *      *      *
CCA GTG CAT AAA CTG TAT ATA GAA TAT GGC ATT CAG AGG ATC ACT TGT
Pro Val His Lys Leu Tyr Ile Glu Tyr Gly Ile Gln Arg Ile Thr Cys>

490     500     510     520
      *      *      *      *      *
CCA AAT GTA GAT GGA TAT TTT CCT TCC AGT GTC AAA CCG ACT ATC ACT
Pro Asn Val Asp Gly Tyr Phe Pro Ser Ser Val Lys Pro Thr Ile Thr>

530     540     550     560     570
      *      *      *      *      *
TGG TAT ATG GGC TGT TAT AAA ATA CAG AAT TTT AAT AAT GTA ATA CCC
Trp Tyr Met Gly Cys Tyr Lys Ile Gln Asn Phe Asn Asn Val Ile Pro>

```

Figure 26B

```

      580      590      600      610      620
      *      *      *      *      *
GAA GGT ATG AAC TTG AGT TTC CTC ATT GCC TTA ATT TCA AAT AAT GGA
Glu Gly Met Asn Leu Ser Phe Leu Ile Ala Leu Ile Ser Asn Asn Gly>

      630      640      650      660      670
      *      *      *      *      *
AAT TAC ACA TGT GTT GTT ACA TAT CCA GAA AAT GGA CGT ACG TTT CAT
Asn Tyr Thr Cys Val Val Thr Tyr Pro Glu Asn Gly Arg Thr Phe His>

      680      690      700      710      720
      *      *      *      *      *
CTC ACC AGG ACT CTG ACT GTA AAG GTA GTA GGC TCT CCA AAA AAT GCA
Leu Thr Arg Thr Leu Thr Val Lys Val Val Gly Ser Pro Lys Asn Ala>

      730      740      750      760
      *      *      *      *      *
GTG CCC CCT GTG ATC CAT TCA CCT AAT GAT CAT GTG GTC TAT GAG AAA
Val Pro Pro Val Ile His Ser Pro Asn Asp His Val Val Tyr Glu Lys>

770      780      790      800      810
      *      *      *      *      *
GAA CCA GGA GAG GAG CTA CTC ATT CCC TGT ACG GTC TAT TTT AGT TTT
Glu Pro Gly Glu Glu Leu Leu Ile Pro Cys Thr Val Tyr Phe Ser Phe>

      820      830      840      850      860
      *      *      *      *      *
CTG ATG GAT TCT CGC AAT GAG GTT TGG TGG ACC ATT GAT GGA AAA AAA
Leu Met Asp Ser Arg Asn Glu Val Trp Trp Thr Ile Asp Gly Lys Lys>

      870      880      890      900      910
      *      *      *      *      *
CCT GAT GAC ATC ACT ATT GAT GTC ACC ATT AAC GAA AGT ATA AGT CAT
Pro Asp Asp Ile Thr Ile Asp Val Thr Ile Asn Glu Ser Ile Ser His>

      920      930      940      950      960
      *      *      *      *      *
AGT AGA ACA GAA GAT GAA ACA AGA ACT CAG ATT TTG AGC ATC AAG AAA
Ser Arg Thr Glu Asp Glu Thr Arg Thr Gln Ile Leu Ser Ile Lys Lys>

      970      980      990      1000
      *      *      *      *      *
GTT ACC TCT GAG GAT CTC AAG CGC AGC TAT GTC TGT CAT GCT AGA AGT
Val Thr Ser Glu Asp Leu Lys Arg Ser Tyr Val Cys His Ala Arg Ser>

1010      1020      1030      1040      1050
      *      *      *      *      *
GCC AAA GGC GAA GTT GCC AAA GCA GCC AAG GTG AAG CAG AAA GTG CCA
Ala Lys Gly Glu Val Ala Lys Ala Ala Lys Val Lys Gln Lys Val Pro>

      1060      1070      1080      1090      1100
      *      *      *      *      *
GCT CCA AGA TAC ACA GTG TCC GGT GGC GCG CCT ATG CTG AGC GAG GCT
Ala Pro Arg Tyr Thr Val Ser Gly Gly Ala Pro Met Leu Ser Glu Ala>

      1110      1120      1130      1140      1150
      *      *      *      *      *
GAT AAA TGC AAG GAA CGT GAA GAA AAA ATA ATT TTA GTG TCA TCT GCA
Asp Lys Cys Lys Glu Arg Glu Glu Lys Ile Ile Leu Val Ser Ser Ala>

      1160      1170      1180      1190      1200
      *      *      *      *      *

```

Figure 26C

```

AAT GAA ATT GAT GTT CGT CCC TGT CCT CTT AAC CCA AAT GAA CAC AAA
Asn Glu Ile Asp Val Arg Pro Cys Pro Leu Asn Pro Asn Glu His Lys>

      1210      1220      1230      1240
      *      *      *      *
GGC ACT ATA ACT TGG TAT AAG GAT GAC AGC AAG ACA CCT GTA TCT ACA
Gly Thr Ile Thr Trp Tyr Lys Asp Asp Ser Lys Thr Pro Val Ser Thr>

1250      1260      1270      1280      1290
*      *      *      *      *
GAA CAA GCC TCC AGG ATT CAT CAA CAC AAA GAG AAA CTT TGG TTT GTT
Glu Gln Ala Ser Arg Ile His Gln His Lys Glu Lys Leu Trp Phe Val>

      1300      1310      1320      1330      1340
      *      *      *      *      *
CCT GCT AAG GTG GAG GAT TCA GGA CAT TAC TAT TGC GTG GTA AGA AAT
Pro Ala Lys Val Glu Asp Ser Gly His Tyr Tyr Cys Val Val Arg Asn>

      1350      1360      1370      1380      1390
      *      *      *      *      *
TCA TCT TAC TGC CTC AGA ATT AAA ATA AGT GCA AAA TTT GTG GAG AAT
Ser Ser Tyr Cys Leu Arg Ile Lys Ile Ser Ala Lys Phe Val Glu Asn>

      1400      1410      1420      1430      1440
      *      *      *      *      *
GAG CCT AAC TTA TGT TAT AAT GCA CAA GCC ATA TTT AAG CAG AAA CTA
Glu Pro Asn Leu Cys Tyr Asn Ala Gln Ala Ile Phe Lys Gln Lys Leu>

      1450      1460      1470      1480
      *      *      *      *
CCC GTT GCA GGA GAC GGA GGA CTT GTG TGC CCT TAT ATG GAG TTT TTT
Pro Val Ala Gly Asp Gly Gly Leu Val Cys Pro Tyr Met Glu Phe Phe>

1490      1500      1510      1520      1530
*      *      *      *      *
AAA AAT GAA AAT AAT GAG TTA CCT AAA TTA CAG TGG TAT AAG GAT TGC
Lys Asn Glu Asn Asn Glu Leu Pro Lys Leu Gln Trp Tyr Lys Asp Cys>

      1540      1550      1560      1570      1580
      *      *      *      *      *
AAA CCT CTA CTT CTT GAC AAT ATA CAC TTT AGT GGA GTC AAA GAT AGG
Lys Pro Leu Leu Leu Asp Asn Ile His Phe Ser Gly Val Lys Asp Arg>

      1590      1600      1610      1620      1630
      *      *      *      *      *
CTC ATC GTG ATG AAT GTG GCT GAA AAG CAT AGA GGG AAC TAT ACT TGT
Leu Ile Val Met Asn Val Ala Glu Lys His Arg Gly Asn Tyr Thr Cys>

      1640      1650      1660      1670      1680
      *      *      *      *      *
CAT GCA TCC TAC ACA TAC TTG GGC AAG CAA TAT CCT ATT ACC CGG GTA
His Ala Ser Tyr Thr Tyr Leu Gly Lys Gln Tyr Pro Ile Thr Arg Val>

      1690      1700      1710      1720
      *      *      *      *
ATA GAA TTT ATT ACT CTA GAG GAA AAC AAA CCC ACA AGG CCT GTG ATT
Ile Glu Phe Ile Thr Leu Glu Glu Asn Lys Pro Thr Arg Pro Val Ile>

1730      1740      1750      1760      1770
*      *      *      *      *
GTG AGC CCA GCT AAT GAG ACA ATG GAA GTA GAC TTG GGA TCC CAG ATA
Val Ser Pro Ala Asn Glu Thr Met Glu Val Asp Leu Gly Ser Gln Ile>

```

Figure 26D

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1780      1790      1800      1810      1820
*         *         *         *         *
CAA TTG ATC TGT AAT GTC ACC GGC CAG TTG AGT GAC ATT GCT TAC TGG
Gln Leu Ile Cys Asn Val Thr Gly Gln Leu Ser Asp Ile Ala Tyr Trp>

1830      1840      1850      1860      1870
*         *         *         *         *
AAG TGG AAT GGG TCA GTA ATT GAT GAA GAT GAC CCA GTG CTA GGG GAA
Lys Trp Asn Gly Ser Val Ile Asp Glu Asp Asp Pro Val Leu Gly Glu>

1880      1890      1900      1910      1920
*         *         *         *         *
GAC TAT TAC AGT GTG GAA AAT CCT GCA AAC AAA AGA AGG AGT ACC CTC
Asp Tyr Tyr Ser Val Glu Asn Pro Ala Asn Lys Arg Arg Ser Thr Leu>

1930      1940      1950      1960
*         *         *         *
ATC ACA GTG CTT AAT ATA TCG GAA ATT GAG AGT AGA TTT TAT AAA CAT
Ile Thr Val Leu Asn Ile Ser Glu Ile Glu Ser Arg Phe Tyr Lys His>

1970      1980      1990      2000      2010
*         *         *         *         *
CCA TTT ACC TGT TTT GCC AAG AAT ACA CAT GGT ATA GAT GCA GCA TAT
Pro Phe Thr Cys Phe Ala Lys Asn Thr His Gly Ile Asp Ala Ala Tyr>

2020      2030      2040      2050      2060
*         *         *         *         *
ATC CAG TTA ATA TAT CCA GTC ACT AAT TCC GGA GAC AAA ACT CAC ACA
Ile Gln Leu Ile Tyr Pro Val Thr Asn Ser Gly Asp Lys Thr His Thr>

2070      2080      2090      2100      2110
*         *         *         *         *
TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC
Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe>

2120      2130      2140      2150      2160
*         *         *         *         *
CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro>

2170      2180      2190      2200
*         *         *         *
GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC
Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val>

2210      2220      2230      2240      2250
*         *         *         *         *
AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA
Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr>

2260      2270      2280      2290      2300
*         *         *         *         *
AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val>

2310      2320      2330      2340      2350
*         *         *         *         *
CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys>

2360      2370      2380      2390      2400

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Figure 26E

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      *      *      *      *      *      *      *      *      *
AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC
Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser>

      2410      2420      2430      2440
      *      *      *      *      *      *      *
AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro>

2450      2460      2470      2480      2490
      *      *      *      *      *      *      *
TCC CGG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC
Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val>

      2500      2510      2520      2530      2540
      *      *      *      *      *      *      *
AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly>

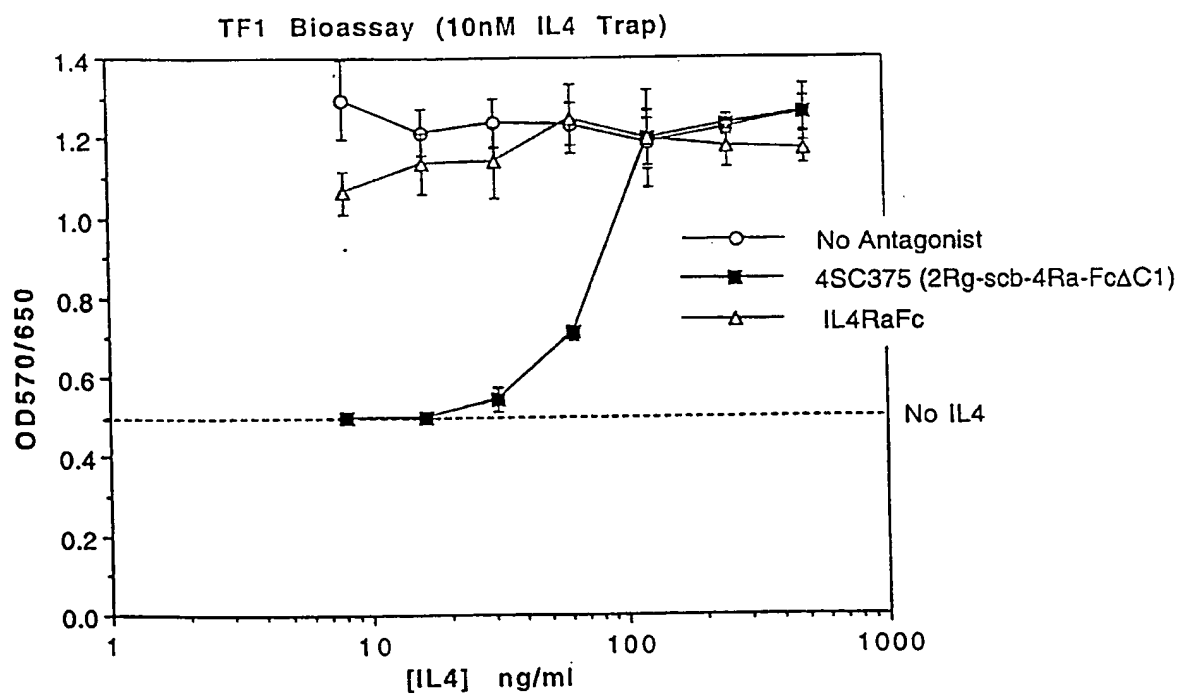
      2550      2560      2570      2580      2590
      *      *      *      *      *      *      *
CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp>

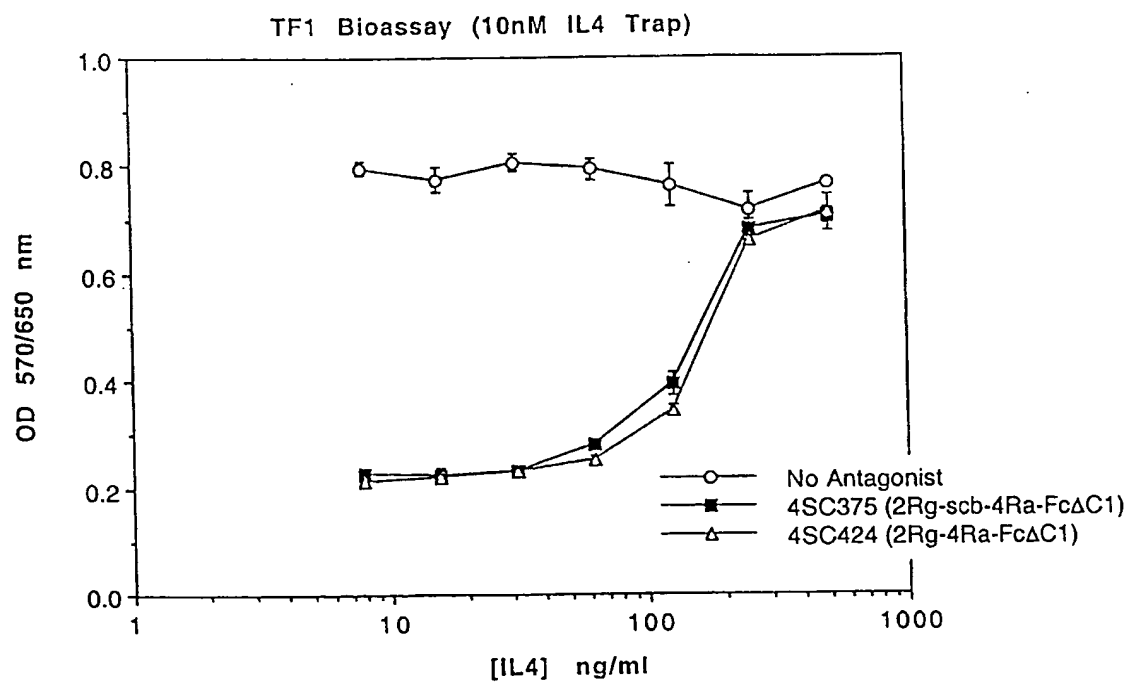
      2600      2610      2620      2630      2640
      *      *      *      *      *      *      *
GGC TCC TTC TTC CTC TAT AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp>

      2650      2660      2670      2680
      *      *      *      *      *      *      *
CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His>

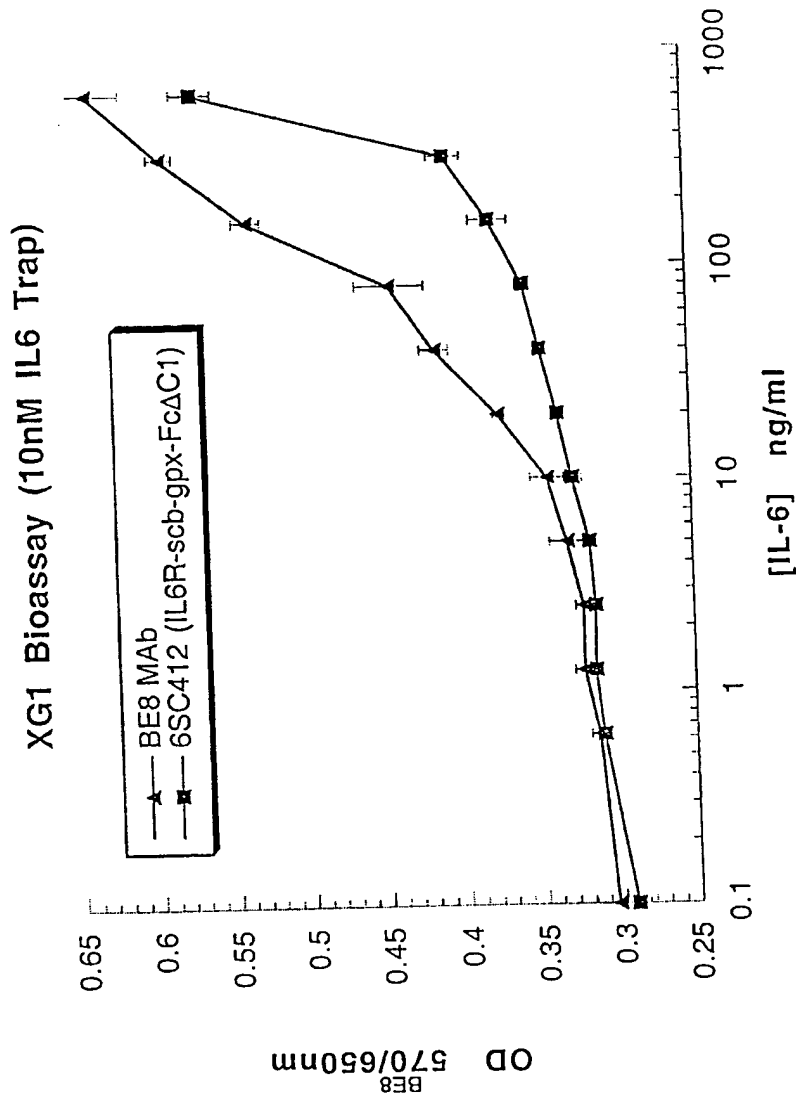
2690      2700      2710      2720      2730
      *      *      *      *      *      *      *
AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys ***>

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Figure 27^{52/74}

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Figure 28

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Figure 29



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Figure 30

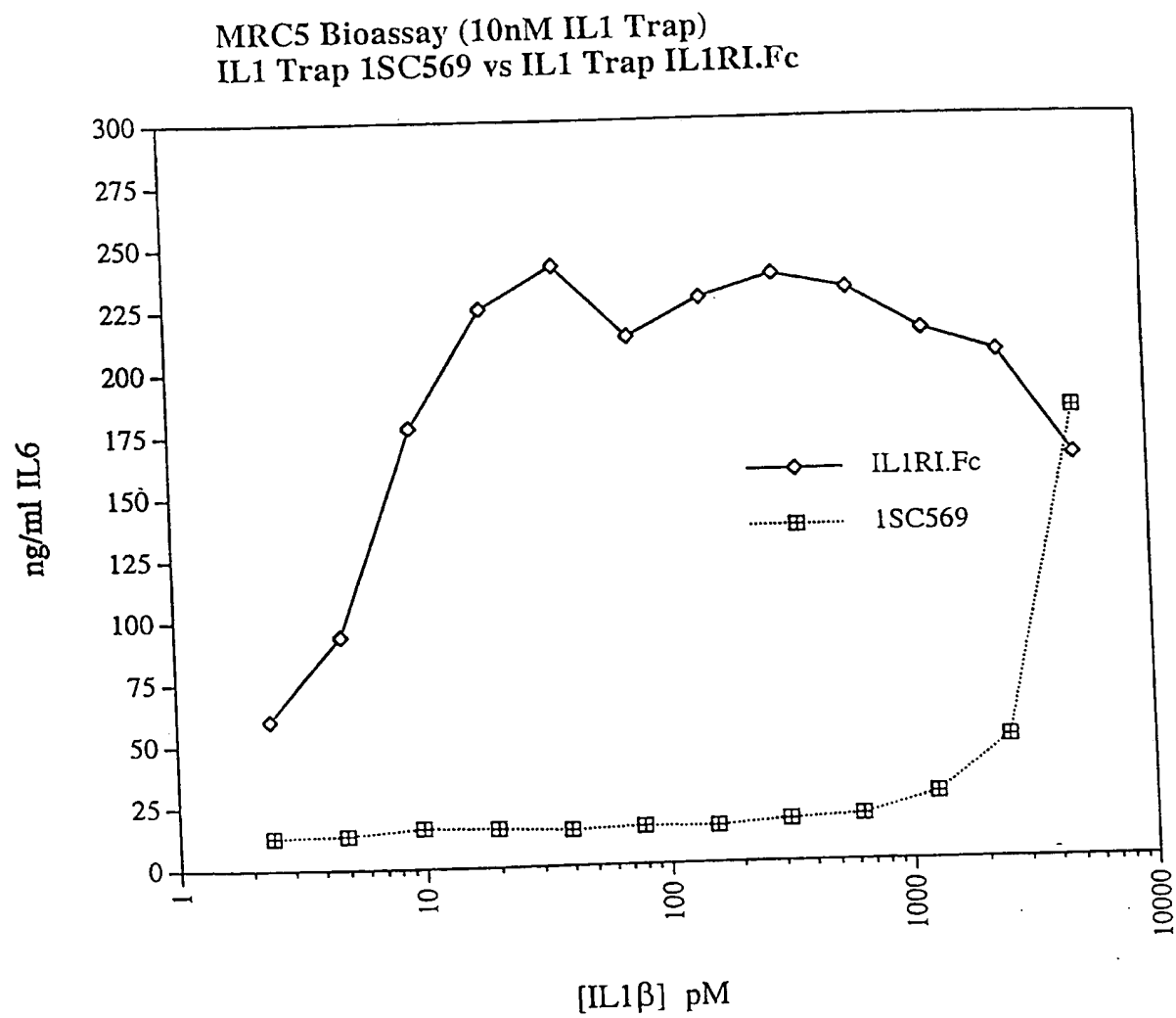


Figure 31A

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      10      20      30      40
      *      *      *      *
ATG GTG TGG CTT TGC TCT GGG CTC CTG TTC CCT GTG AGC TGC CTG GTC
TAC CAC ACC GAA ACG AGA CCC GAG GAC AAG GGA CAC TCG ACG GAC CAG
Met Val Trp Leu Cys Ser Gly Leu Leu Phe Pro Val Ser Cys Leu Val>

50      60      70      80      90
      *      *      *      *      *
CTG CTG CAG GTG GCA AGC TCT GGG AAC ATG AAG GTC TTG CAG GAG CCC
GAC GAC GTC CAC CGT TCG AGA CCC TTG TAC TTC CAG AAC GTC CTC GGG
Leu Leu Gln Val Ala Ser Ser Gly Asn Met Lys Val Leu Gln Glu Pro>

100     110     120     130     140
      *      *      *      *      *
ACC TGC GTC TCC GAC TAC ATG AGC ATC TCT ACT TGC GAG TGG AAG ATG
TGG ACG CAG AGG CTG ATG TAC TCG TAG AGA TGA ACG CTC ACC TTC TAC
Thr Cys Val Ser Asp Tyr Met Ser Ile Ser Thr Cys Glu Trp Lys Met>

150     160     170     180     190
      *      *      *      *      *
AAT GGT CCC ACC AAT TGC AGC ACC GAG CTC CGC CTG TTG TAC CAG CTG
TTA CCA GGG TGG TTA ACG TCG TGG CTC GAG GCG GAC AAC ATG GTC GAC
Asn Gly Pro Thr Asn Cys Ser Thr Glu Leu Arg Leu Leu Tyr Gln Leu>

200     210     220     230     240
      *      *      *      *      *
GTT TTT CTG CTC TCC GAA GCC CAC ACG TGT ATC CCT GAG AAC AAC GGA
CAA AAA GAC GAG AGG CTT CGG GTG TGC ACA TAG GGA CTC TTG TTG CCT
Val Phe Leu Leu Ser Glu Ala His Thr Cys Ile Pro Glu Asn Asn Gly>

250     260     270     280
      *      *      *      *      *
GGC GCG GGG TGC GTG TGC CAC CTG CTC ATG GAT GAC GTG GTC AGT GCG
CCG CGC CCC ACG CAC ACG GTG GAC GAG TAC CTA CTG CAC CAG TCA CGC
Gly Ala Gly Cys Val Cys His Leu Leu Met Asp Asp Val Val Ser Ala>

290     300     310     320     330
      *      *      *      *      *
GAT AAC TAT ACA CTG GAC CTG TGG GCT GGG CAG CAG CTG CTG TGG AAG
CTA TTG ATA TGT GAC CTG GAC ACC CGA CCC GTC GTC GAC GAC ACC TTC
Asp Asn Tyr Thr Leu Asp Leu Trp Ala Gly Gln Gln Leu Leu Trp Lys>

340     350     360     370     380
      *      *      *      *      *
GGC TCC TTC AAG CCC AGC GAG CAT GTG AAA CCC AGG GCC CCA GGA AAC
CCG AGG AAG TTC GGG TCG CTC GTA CAC TTT GGG TCC CGG GGT CCT TTG
Gly Ser Phe Lys Pro Ser Glu His Val Lys Pro Arg Ala Pro Gly Asn>

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Figure 31B

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      390      400      410      420      430
      *      *      *      *      *
CTG ACA GTT CAC ACC AAT GTC TCC GAC ACT CTG CTG CTG ACC TGG AGC
GAC TGT CAA GTG TGG TTA CAG AGG CTG TGA GAC GAC GAC TGG ACC TCG
Leu Thr Val His Thr Asn Val Ser Asp Thr Leu Leu Leu Thr Trp Ser>

      440      450      460      470      480
      *      *      *      *      *
AAC CCG TAT CCC CCT GAC AAT TAC CTG TAT AAT CAT CTC ACC TAT GCA
TTG GGC ATA GGG GGA CTG TTA ATG GAC ATA TTA GTA GAG TGG ATA CGT
Asn Pro Tyr Pro Pro Asp Asn Tyr Leu Tyr Asn His Leu Thr Tyr Ala>

      490      500      510      520
      *      *      *      *
GTC AAC ATT TGG AGT GAA AAC GAC CCG GCA GAT TTC AGA ATC TAT AAC
CAG TTG TAA ACC TCA CTT TTG CTG GGC CGT CTA AAG TCT TAG ATA TTG
Val Asn Ile Trp Ser Glu Asn Asp Pro Ala Asp Phe Arg Ile Tyr Asn>

530      540      550      560      570
      *      *      *      *      *
GTG ACC TAC CTA GAA CCC TCC CTC CGC ATC GCA GCC AGC ACC CTG AAG
CAC TGG ATG GAT CTT GGG AGG GAG GCG TAG CGT CGG TCG TGG GAC TTC
Val Thr Tyr Leu Glu Pro Ser Leu Arg Ile Ala Ala Ser Thr Leu Lys>

      580      590      600      610      620
      *      *      *      *      *
TCT GGG ATT TCC ATG AGG GCA CGG GTG AGG GCC TGG GCT CAG AGC TAT
AGA CCC TAA AGG ATG TCC CGT GCC CAC TCC CGG ACC CGA GTC TCG ATA
Ser Gly Ile Ser Tyr Arg Ala Arg Val Arg Ala Trp Ala Gln Ser Tyr>

      630      640      650      660      670
      *      *      *      *      *
AAC ACC ACC TGG AGT GAG TGG AGC CCC AGC ACC AAG TGG CAC AAC TCC
TTG TGG TGG ACC TCA CTC ACC TCG GGG TCG TGG TTC ACC GTG TTG AGG
Asn Thr Thr Trp Ser Glu Trp Ser Pro Ser Thr Lys Trp His Asn Ser>

      680      690      700      710      720
      *      *      *      *      *
TAC AGG GAG CCC TTC GAG CAG TCC GGT GGG GGC GGG GGC GCC GCG CCT
ATG TCC CTC GGG AAG CTC GTC AGG CCA CCC CCG CCC CCG CGG CGC GGA
Tyr Arg Glu Pro Phe Glu Gln Ser Gly Gly Gly Gly Gly Ala Ala Pro>

      730      740      750      760
      *      *      *      *
ACG GAA ACT CAG CCA CCT GTG ACA AAT TTG AGT GTC TCT GTT GAA AAC
TGC CTT TGA GTC GGT GGA CAC TGT TTA AAC TCA CAG AGA CAA CTT TTG
Thr Glu Thr Gln Pro Pro Val Thr Asn Leu Ser Val Ser Val Glu Asn>

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Figure 31C

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770      780      790      800      810
*      *      *      *      *
CTC TGC ACA GTA ATA TGG ACA TGG AAT CCA CCC GAG GGA GCC AGC TCA
GAG ACG TGT CAT TAT ACC TGT ACC TTA GGT GGG CTC CCT CGG TCG AGT
Leu Cys Thr Val Ile Trp Thr Trp Asn Pro Pro Glu Gly Ala Ser Ser>

      820      830      840      850      860
*      *      *      *      *
AAT TGT AGT CTA TGG TAT TTT AGT CAT TTT GGC GAC AAA CAA GAT AAG
TTA ACA TCA GAT ACC ATA AAA TCA GTA AAA CCG CTG TTT GTT CTA TTC
Asn Cys Ser Leu Trp Tyr Phe Ser His Phe Gly Asp Lys Gln Asp Lys>

      870      880      890      900      910
*      *      *      *      *
AAA ATA GCT CCG GAA ACT CGT CGT TCA ATA GAA GTA CCC CTG AAT GAG
TTT TAT CGA GGC CTT TGA GCA GCA AGT TAT CTT CAT GGG GAC TTA CTC
Lys Ile Ala Pro Glu Thr Arg Arg Ser Ile Glu Val Pro Leu Asn Glu>

      920      930      940      950      960
*      *      *      *      *
AGG ATT TGT CTG CAA GTG GGG TCC CAG TGT AGC ACC AAT GAG AGT GAG
TCC TAA ACA GAC GTT CAC CCC AGG GTC ACA TCG TGG TTA CTC TCA CTC
Arg Ile Cys Leu Gln Val Gly Ser Gln Cys Ser Thr Asn Glu Ser Glu>

      970      980      990      1000
*      *      *      *
AAG CCT AGC ATT TTG GTT GAA AAA TGC ATC TCA CCC CCA GAA GGT GAT
TTC GGA TCG TAA AAC CAA CTT TTT ACG TAG AGT GGG GGT CTT CCA CTA
Lys Pro Ser Ile Leu Val Glu Lys Cys Ile Ser Pro Pro Glu Gly Asp>

1010      1020      1030      1040      1050
*      *      *      *      *
CCT GAG TCT GCT GTG ACT GAG CTT CAA TGC ATT TGG CAC AAC CTG AGC
GGA CTC AGA CGA CAC TGA CTC GAA GTT ACG TAA ACC GTG TTG GAC TCG
Pro Glu Ser Ala Val Thr Glu Leu Gln Cys Ile Trp His Asn Leu Ser>

      1060      1070      1080      1090      1100
*      *      *      *      *
TAC ATG AAG TGT TCT TGG CTC CCT GGA AGG AAT ACC AGT CCC GAC ACT
ATG TAC TTC ACA AGA ACC GAG GGA CCT TCC TTA TGG TCA GGG CTG TGA
Tyr Met Lys Cys Ser Trp Leu Pro Gly Arg Asn Thr Ser Pro Asp Thr>

      1110      1120      1130      1140      1150
*      *      *      *      *
AAC TAT ACT CTC TAC TAT TGG CAC AGA AGC CTG GAA AAA ATT CAT CAA
TTG ATA TGA GAG ATG ATA ACC GTG TCT TCG GAC CTT TTT TAA GTA GTT
Asn Tyr Thr Leu Tyr Tyr Trp His Arg Ser Leu Glu Lys Ile His Gln>

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Figure 31D

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      1160      1170      1180      1190      1200
      *        *        *        *        *
TGT GAA AAC ATC TTT AGA GAA GGC CAA TAC TTT GGT TGT TCC TTT GAT
ACA CTT TTG TAG AAA TCT CTT CCG GTT ATG AAA CCA ACA AGG AAA CTA
Cys Glu Asn Ile Phe Arg Glu Gly Gln Tyr Phe Gly Cys Ser Phe Asp>

      1210      1220      1230      1240
      *        *        *        *        *
CTG ACC AAA GTG AAG GAT TCC AGT TTT GAA CAA CAC AGT GTC CAA ATA
GAC TGG TTT CAC TTC CTA AGG TCA AAA CTT GTT GTG TCA CAG GTT TAT
Leu Thr Lys Val Lys Asp Ser Ser Phe Glu Gln His Ser Val Gln Ile>

1250      1260      1270      1280      1290
      *        *        *        *        *
ATG GTC AAG GAT AAT GCA GGA AAA ATT AAA CCA TCC TTC AAT ATA GTG
TAC CAG TTC CTA TTA CGT CCT TTT TAA TTT GGT AGG AAG TTA TAT CAC
Met Val Lys Asp Asn Ala Gly Lys Ile Lys Pro Ser Phe Asn Ile Val>

      1300      1310      1320      1330      1340
      *        *        *        *        *
CCT TTA ACT TCC CGT GTG AAA CCT GAT CCT CCA CAT ATT AAA AAC CTC
GGA AAT TGA AGG GCA CAC TTT GGA CTA GGA GGT GTA TAA TTT TTG GAG
Pro Leu Thr Ser Arg Val Lys Pro Asp Pro Pro His Ile Lys Asn Leu>

      1350      1360      1370      1380      1390
      *        *        *        *        *
TCC TTC CAC AAT GAT GAC CTA TAT GTG CAA TGG GAG AAT CCA CAG AAT
AGG AAG GTG TTA CTA CTG GAT ATA CAC GTT ACC CTC TTA GGT GTC TTA
Ser Phe His Asn Asp Asp Leu Tyr Val Gln Trp Glu Asn Pro Gln Asn>

      1400      1410      1420      1430      1440
      *        *        *        *        *
TTT ATT AGC AGA TGC CTA TTT TAT GAA GTA GAA GTC AAT AAC AGC CAA
AAA TAA TCG TCT ACG GAT AAA ATA CTT CAT CTT CAG TTA TTG TCG GTT
Phe Ile Ser Arg Cys Leu Phe Tyr Glu Val Glu Val Asn Asn Ser Gln>

      1450      1460      1470      1480
      *        *        *        *        *
ACT GAG ACA CAT AAT GTT TTC TAC GTC CAA GAG GCT AAA TGT GAG AAT
TGA CTC TGT GTA TTA CAA AAG ATG CAG GTT CTC CGA TTT ACA CTC TTA
Thr Glu Thr His Asn Val Phe Tyr Val Gln Glu Ala Lys Cys Glu Asn>

1490      1500      1510      1520      1530
      *        *        *        *        *
CCA GAA TTT GAG AGA AAT GTG GAG AAT ACA TCT TGT TTC ATG GTC CCT
GGT CTT AAA CTC TCT TTA CAC CTC TTA TGT AGA ACA AAG TAC CAG GGA
Pro Glu Phe Glu Arg Asn Val Glu Asn Thr Ser Cys Phe Met Val Pro>

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Figure 31E

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1540      1550      1560      1570      1580
*      *      *      *      *
GGT GTT CTT CCT GAT ACT TTG AAC ACA GTC AGA ATA AGA GTC AAA ACA
CCA CAA GAA GGA CTA TGA AAC TTG TGT CAG TCT TAT TCT CAG TTT TGT
Gly Val Leu Pro Asp Thr Leu Asn Thr Val Arg Ile Arg Val Lys Thr>

      1590      1600      1610      1620      1630
*      *      *      *      *
AAT AAG TTA TGC TAT GAG GAT GAC AAA CTC TGG AGT AAT TGG AGC CAA
TTA TTC AAT ACG ATA CTC CTA CTG TTT GAG ACC TCA TTA ACC TCG GTT
Asn Lys Leu Cys Tyr Glu Asp Asp Lys Leu Trp Ser Asn Trp Ser Gln>

      1640      1650      1660      1670      1680
*      *      *      *      *
GAA ATG AGT ATA GGT AAG AAG CGC AAT TCC ACA ACC GGA GAC AAA ACT
CTT TAC TCA TAT CCA TTC TTC GCG TTA AGG TGT TGG CCT CTG TTT TGA
Glu Met Ser Ile Gly Lys Lys Arg Asn Ser Thr Thr Gly Asp Lys Thr>

      1690      1700      1710      1720
*      *      *      *      *
CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA
GTG TGT ACG GGT GGC ACG GGT CGT GGA CTT GAG GAC CCC CCT GGC AGT
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser>

1730      1740      1750      1760      1770
*      *      *      *      *
GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG
CAG AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG GAG TAC TAG AGG GCC
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg>

      1780      1790      1800      1810      1820
*      *      *      *      *
ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT
TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC TCG GTG CTT CTG GGA
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro>

      1830      1840      1850      1860      1870
*      *      *      *      *
GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC
CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA TTA CGG
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala>

      1880      1890      1900      1910      1920
*      *      *      *      *
AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC
TTC TGT TTC GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA CAC CAG
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val>

```

Figure 31F

	1930	1940	1950	1960	
	*	*	*	*	*
	AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC				
	TCG CAG GAG TGG CAG GAC GTG GTC CTG ACC GAC TTA CCG TTC CTC ATG				
	Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr>				
1970	1980	1990	2000	2010	
*	*	*	*	*	*
AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC					
TTC ACG TTC CAG AGG TTG TTT CGG GAG GGT CGG GGG TAG CTC TTT TGG					
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr>					
2020	2030	2040	2050	2060	
*	*	*	*	*	*
ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG					
TAG AGG TTT CGG TTT CCC GTC GGG GCT CTT GGT GTC CAC ATG TGG GAC					
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu>					
2070	2080	2090	2100	2110	
*	*	*	*	*	*
CCC CCA TCC CGG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC					
GGG GGT AGG GCC CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC TGG ACG					
Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys>					
2120	2130	2140	2150	2160	
*	*	*	*	*	*
CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC					
GAC CAG TTT CCG AAG ATA GGG TCG CTG TAG CGG CAC CTC ACC CTC TCG					
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser>					
2170	2180	2190	2200		
*	*	*	*	*	*
AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC					
TTA CCC GTC GGC CTC TTG TTG ATG TTC TGG TGC GGA GGG CAC GAC CTG					
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp>					
2210	2220	2230	2240	2250	
*	*	*	*	*	*
TCC GAC GGC TCC TTC TTC CTC TAT AGC AAG CTC ACC GTG GAC AAG AGC					
AGG CTG CCG AGG AAG AAG GAG ATA TCG TTC GAG TGG CAC CTG TTC TCG					
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser>					
2260	2270	2280	2290	2300	
*	*	*	*	*	*
AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT					
TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG CAC TAC GTA CTC CGA					
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala>					

Figure 31G

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      2310      2320      2330      2340      2350
*      *      *      *      *      *      *      *      *
CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA
GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC CCA TTT
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys>

*
TGA
ACT
***>
```


Figure 32A

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      10      20      30      40
      *      *      *      *      *
ATG GTG TGG CCG GCG CGG CTC TGC GGG CTG TGG GCG CTG CTG CTC TGC
TAC CAC ACC GGC CGC GCC GAG ACG CCC GAC ACC CGC GAC GAC GAG ACG
Met Val Trp Pro Ala Arg Leu Cys Gly Leu Trp Ala Leu Leu Leu Cys>

50      60      70      80      90
      *      *      *      *      *
GCC GGC GGC GGG GGC GGG GGC GGG GGC GCC GCG CCT ACG GAA ACT CAG
CGG CCG CCG CCC CCG CCC CCG CCC CCG CGG CGC GGA TGC CTT TGA GTC
Ala Gly Gly Gly Gly Gly Gly Gly Gly Ala Ala Pro Thr Glu Thr Gln>

100     110     120     130     140
      *      *      *      *      *
CCA CCT GTG ACA AAT TTG AGT GTC TCT GTT GAA AAC CTC TGC ACA GTA
GGT GGA CAC TGT TTA AAC TCA CAG AGA CAA CTT TTG GAG ACG TGT CAT
Pro Pro Val Thr Asn Leu Ser Val Ser Val Glu Asn Leu Cys Thr Val>

150     160     170     180     190
      *      *      *      *      *
ATA TGG ACA TGG AAT CCA CCC GAG GGA GCC AGC TCA AAT TGT AGT CTA
TAT ACC TGT ACC TTA GGT GGG CTC CCT CGG TCG AGT TTA ACA TCA GAT
Ile Trp Thr Trp Asn Pro Pro Glu Gly Ala Ser Ser Asn Cys Ser Leu>

200     210     220     230     240
      *      *      *      *      *
TGG TAT TTT AGT CAT TTT GGC GAC AAA CAA GAT AAG AAA ATA GCT CCG
ACC ATA AAA TCA GTA AAA CCG CTG TTT GTT CTA TTC TTT TAT CGA GGC
Trp Tyr Phe Ser His Phe Gly Asp Lys Gln Asp Lys Lys Ile Ala Pro>

250     260     270     280
      *      *      *      *      *
GAA ACT CGT CGT TCA ATA GAA GTA CCC CTG AAT GAG AGG ATT TGT CTG
CTT TGA GCA GCA AGT TAT CTT CAT GGG GAC TTA CTC TCC TAA ACA GAC
Glu Thr Arg Arg Ser Ile Glu Val Pro Leu Asn Glu Arg Ile Cys Leu>

290     300     310     320     330
      *      *      *      *      *
CAA GTG GGG TCC CAG TGT AGC ACC AAT GAG AGT GAG AAG CCT AGC ATT
GTT CAC CCC AGG GTC ACA TCG TGG TTA CTC TCA CTC TTC GGA TCG TAA
Gln Val Gly Ser Gln Cys Ser Thr Asn Glu Ser Glu Lys Pro Ser Ile>

340     350     360     370     380
      *      *      *      *      *
TTG GTT GAA AAA TGC ATC TCA CCC CCA GAA GGT GAT CCT GAG TCT GCT
AAC CAA CTT TTT ACG TAG AGT GGG GGT CTT CCA CTA GGA CTC AGA CGA
Leu Val Glu Lys Cys Ile Ser Pro Pro Glu Gly Asp Pro Glu Ser Ala>

```

Figure 32B

```

      390      400      410      420      430
*      *      *      *      *      *      *      *
GTG ACT GAG CTT CAA TGC ATT TGG CAC AAC CTG AGC TAC ATG AAG TGT
CAC TGA CTC GAA GTT ACG TAA ACC GTG TTG GAC TCG ATG TAC TTC ACA
Val Thr Glu Leu Gln Cys Ile Trp His Asn Leu Ser Tyr Met Lys Cys>

      440      450      460      470      480
*      *      *      *      *      *      *      *
TCT TGG CTC CCT GGA AGG AAT ACC AGT CCC GAC ACT AAC TAT ACT CTC
AGA ACC GAG GGA CCT TCC TTA TGG TCA GGG CTG TGA TTG ATA TGA GAG
Ser Trp Leu Pro Gly Arg Asn Thr Ser Pro Asp Thr Asn Tyr Thr Leu>

      490      500      510      520
*      *      *      *      *      *      *      *
TAC TAT TGG CAC AGA AGC CTG GAA AAA ATT CAT CAA TGT GAA AAC ATC
ATG ATA ACC GTG TCT TCG GAC CTT TTT TAA GTA GTT ACA CTT TTG TAG
Tyr Tyr Trp His Arg Ser Leu Glu Lys Ile His Gln Cys Glu Asn Ile>

530      540      550      560      570
*      *      *      *      *      *      *      *
TTT AGA GAA GGC CAA TAC TTT GGT TGT TCC TTT GAT CTG ACC AAA GTG
AAA TCT CTT CCG GTT ATG AAA CCA ACA AGG AAA CTA GAC TGG TTT CAC
Phe Arg Glu Gly Gln Tyr Phe Gly Cys Ser Phe Asp Leu Thr Lys Val>

      580      590      600      610      620
*      *      *      *      *      *      *      *
AAG GAT TCC AGT TTT GAA CAA CAC AGT GTC CAA ATA ATG GTC AAG GAT
TTC CTA AGG TCA AAA CTT GTT GTG TCA CAG GTT TAT TAC CAG TTC CTA
Lys Asp Ser Ser Phe Glu Gln His Ser Val Gln Ile Met Val Lys Asp>

      630      640      650      660      670
*      *      *      *      *      *      *      *
AAT GCA GGA AAA ATT AAA CCA TCC TTC AAT ATA GTG CCT TTA ACT TCC
TTA CGT CCT TTT TAA TTT GGT AGG AAG TTA TAT CAC GGA AAT TGA AGG
Asn Ala Gly Lys Ile Lys Pro Ser Phe Asn Ile Val Pro Leu Thr Ser>

      680      690      700      710      720
*      *      *      *      *      *      *      *
CGT GTG AAA CCT GAT CCT CCA CAT ATT AAA AAC CTC TCC TTC CAC AAT
GCA CAC TTT GGA CTA GGA GGT GTA TAA TTT TTG GAG AGG AAG GTG TTA
Arg Val Lys Pro Asp Pro Pro His Ile Lys Asn Leu Ser Phe His Asn>

      730      740      750      760
*      *      *      *      *      *      *      *
GAT GAC CTA TAT GTG CAA TGG GAG AAT CCA CAG AAT TTT ATT AGC AGA
CTA CTG GAT ATA CAC GTT ACC CTC TTA GGT GTC TTA AAA TAA TCG TCT
Asp Asp Leu Tyr Val Gln Trp Glu Asn Pro Gln Asn Phe Ile Ser Arg>

```

Figure 32C

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770      780      790      800      810
*        *        *        *        *
TGC CTA TTT TAT GAA GTA GAA GTC AAT AAC AGC CAA ACT GAG ACA CAT
ACG GAT AAA ATA CTT CAT CTT CAG TTA TTG TCG GTT TGA CTC TGT GTA
Cys Leu Phe Tyr Glu Val Glu Val Asn Asn Ser Gln Thr Glu Thr His>

      820      830      840      850      860
*        *        *        *        *
AAT GTT TTC TAC GTC CAA GAG GCT AAA TGT GAG AAT CCA GAA TTT GAG
TTA CAA AAG ATG CAG GTT CTC CGA TTT ACA CTC TTA GGT CTT AAA CTC
Asn Val Phe Tyr Val Gln Glu Ala Lys Cys Glu Asn Pro Glu Phe Glu>

      870      880      890      900      910
*        *        *        *        *
AGA AAT GTG GAG AAT ACA TCT TGT TTC ATG GTC CCT GGT GTT CTT CCT
TCT TTA CAC CTC TTA TGT AGA ACA AAG TAC CAG GGA CCA CAA GAA GGA
Arg Asn Val Glu Asn Thr Ser Cys Phe Met Val Pro Gly Val Leu Pro>

      920      930      940      950      960
*        *        *        *        *
GAT ACT TTG AAC ACA GTC AGA ATA AGA GTC AAA ACA AAT AAG TTA TGC
CTA TGA AAC TTG TGT CAG TCT TAT TCT CAG TTT TGT TTA TTC AAT ACG
Asp Thr Leu Asn Thr Val Arg Ile Arg Val Lys Thr Asn Lys Leu Cys>

      970      980      990      1000
*        *        *        *
TAT GAG GAT GAC AAA CTC TGG AGT AAT TGG AGC CAA GAA ATG AGT ATA
ATA CTC CTA CTG TTT GAG ACC TCA TTA ACC TCG GTT CTT TAC TCA TAT
Tyr Glu Asp Asp Lys Leu Trp Ser Asn Trp Ser Gln Glu Met Ser Ile>

1010      1020      1030      1040      1050
*        *        *        *        *
GGT AAG AAG CGC AAT TCC ACA GGC GCG CCT AGT GGT GGA GGT GGC CGG
CCA TTC TTC GCG TTA AGG TGT CCG CGC GGA TCA CCA CCT CCA CCG GCC
Gly Lys Lys Arg Asn Ser Thr Gly Ala Pro Ser Gly Gly Gly Gly Arg>

      1060      1070      1080      1090      1100
*        *        *        *        *
CCC GCA AGC TCT GGG AAC ATG AAG GTC TTG CAG GAG CCC ACC TGC GTC
GGG CGT TCG AGA CCC TTG TAC TTC CAG AAC GTC CTC GGG TGG ACG CAG
Pro Ala Ser Ser Gly Asn Met Lys Val Leu Gln Glu Pro Thr Cys Val>

      1110      1120      1130      1140      1150
*        *        *        *        *
TCC GAC TAC ATG AGC ATC TCT ACT TGC GAG TGG AAG ATG AAT GGT CCC
AGG CTG ATG TAC TCG TAG AGA TGA ACG CTC ACC TTC TAC TTA CCA GGG
Ser Asp Tyr Met Ser Ile Ser Thr Cys Glu Trp Lys Met Asn Gly Pro>

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Figure 32D

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      1160      1170      1180      1190      1200
      *        *        *        *        *
ACC AAT TGC AGC ACC GAG CTC CGC CTG TTG TAC CAG CTG GTT TTT CTG
TGG TTA ACG TCG TGG CTC GAG GCG GAC AAC ATG GTC GAC CAA AAA GAC
Thr Asn Cys Ser Thr Glu Leu Arg Leu Leu Tyr Gln Leu Val Phe Leu>

      1210      1220      1230      1240
      *        *        *        *        *
CTC TCC GAA GCC CAC ACG TGT ATC CCT GAG AAC AAC GGA GGC GCG GGG
GAG AGG CTT CGG GTG TGC ACA TAG GGA CTC TTG TTG CCT CCG CGC CCC
Leu Ser Glu Ala His Thr Cys Ile Pro Glu Asn Asn Gly Gly Ala Gly>

1250      1260      1270      1280      1290
      *        *        *        *        *
TGC GTG TGC CAC CTG CTC ATG GAT GAC GTG GTC AGT GCG GAT AAC TAT
ACG CAC ACG GTG GAC GAG TAC CTA CTG CAC CAG TCA CGC CTA TTG ATA
Cys Val Cys His Leu Leu Met Asp Asp Val Val Ser Ala Asp Asn Tyr>

      1300      1310      1320      1330      1340
      *        *        *        *        *
ACA CTG GAC CTG TGG GCT GGG CAG CAG CTG CTG TGG AAG GGC TCC TTC
TGT GAC CTG GAC ACC CGA CCC GTC GTC GAC GAC ACC TTC CCG AGG AAG
Thr Leu Asp Leu Trp Ala Gly Gln Gln Leu Leu Trp Lys Gly Ser Phe>

      1350      1360      1370      1380      1390
      *        *        *        *        *
AAG CCC AGC GAG CAT GTG AAA CCC AGG GCC CCA GGA AAC CTG ACA GTT
TTC GGG TCG CTC GTA CAC TTT GGG TCC CGG GGT CCT TTG GAC TGT CAA
Lys Pro Ser Glu His Val Lys Pro Arg Ala Pro Gly Asn Leu Thr Val>

      1400      1410      1420      1430      1440
      *        *        *        *        *
CAC ACC AAT GTC TCC GAC ACT CTG CTG CTG ACC TGG AGC AAC CCG TAT
GTG TGG TTA CAG AGG CTG TGA GAC GAC GAC TGG ACC TCG TTG GGC ATA
His Thr Asn Val Ser Asp Thr Leu Leu Thr Trp Ser Asn Pro Tyr>

      1450      1460      1470      1480
      *        *        *        *        *
CCC CCT GAC AAT TAC CTG TAT AAT CAT CTC ACC TAT GCA GTC AAC ATT
GGG GGA CTG TTA ATG GAC ATA TTA GTA GAG TGG ATA CGT CAG TTG TAA
Pro Pro Asp Asn Tyr Leu Tyr Asn His Leu Thr Tyr Ala Val Asn Ile>

1490      1500      1510      1520      1530
      *        *        *        *        *
TGG AGT GAA AAC GAC CCG GCA GAT TTC AGA ATC TAT AAC GTG ACC TAC
ACC TCA CTT TTG CTG GGC CGT CTA AAG TCT TAG ATA TTG CAC TGG ATG
Trp Ser Glu Asn Asp Pro Ala Asp Phe Arg Ile Tyr Asn Val Thr Tyr>

```

Figure 32E

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1540      1550      1560      1570      1580
  *      *      *      *      *
CTA GAA CCC TCC CTC CGC ATC GCA GCC AGC ACC CTG AAG TCT GGG ATT
GAT CTT GGG AGG GAG GCG TAG CGT CGG TCG TGG GAC TTC AGA CCC TAA
Leu Glu Pro Ser Leu Arg Ile Ala Ala Ser Thr Leu Lys Ser Gly Ile>

      1590      1600      1610      1620      1630
  *      *      *      *      *
TCC TAC AGG GCA CGG GTG AGG GCC TGG GCT CAG TGC TAT AAC ACC ACC
AGG ATG TCC CGT GCC CAC TCC CGG ACC CGA GTC ACG ATA TTG TGG TGG
Ser Tyr Arg Ala Arg Val Arg Ala Trp Ala Gln Cys Tyr Asn Thr Thr>

      1640      1650      1660      1670      1680
  *      *      *      *      *
TGG AGT GAG TGG AGC CCC AGC ACC AAG TGG CAC AAC TCC TAC AGG GAG
ACC TCA CTC ACC TCG GGG TCG TGG TTC ACC GTG TTG AGG ATG TCC CTC
Trp Ser Glu Trp Ser Pro Ser Thr Lys Trp His Asn Ser Tyr Arg Glu>

      1690      1700      1710      1720
  *      *      *      *      *
CCC TTC GAG CAG TCC GGA GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA
GGG AAG CTC GTC AGG CCT CTG TTT TGA GTG TGT ACG GGT GGC ACG GGT
Pro Phe Glu Gln Ser Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro>

1730      1740      1750      1760      1770
  *      *      *      *      *
GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA
CGT GGA CTT GAG GAC CCC CCT GGC AGT CAG AAG GAG AAG GGG GGT TTT
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys>

      1780      1790      1800      1810      1820
  *      *      *      *      *
CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG
GGG TTC CTG TGG GAG TAC TAG AGG GCC TGG GGA CTC CAG TGT ACG CAC
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val>

      1830      1840      1850      1860      1870
  *      *      *      *      *
GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC
CAC CAC CTG CAC TCG GTG CTT CTG GGA CTC CAG TTC AAG TTG ACC ATG
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr>

      1880      1890      1900      1910      1920
  *      *      *      *      *
GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG
CAC CTG CCG CAC CTC CAC GTA TTA CGG TTC TGT TTC GGC GCC CTC CTC
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu>

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Figure 32F

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      1930      1940      1950      1960
      *        *        *        *
CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC
GTC ATG TTG TCG TGC ATG GCA CAC CAG TCG CAG GAG TGG CAG GAC GTG
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His>

1970      1980      1990      2000      2010
      *        *        *        *        *
CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA
GTC CTG ACC GAC TTA CCG TTC CTC ATG TTC ACG TTC CAG AGG TTG TTT
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys>

      2020      2030      2040      2050      2060
      *        *        *        *        *
GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG
CGG GAG GGT CGG GGG TAG CTC TTT TGG TAG AGG TTT CGG TTT CCC GTC
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln>

      2070      2080      2090      2100      2110
      *        *        *        *        *
CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAG GAG ATG
GGG GCT CTT GGT GTC CAC ATG TGG GAC GGG GGT AGG GCC CTC CTC TAC
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met>

      2120      2130      2140      2150      2160
      *        *        *        *        *
ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC
TGG TTC TTG GTC CAG TCG GAC TGG ACG GAC CAG TTT CCG AAG ATA GGG
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro>

      2170      2180      2190      2200
      *        *        *        *
AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC
TCG CTG TAG CGG CAC CTC ACC CTC TCG TTA CCC GTC GGC CTC TTG TTG
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn>

2210      2220      2230      2240      2250
      *        *        *        *        *
TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC
ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG CCG AGG AAG AAG GAG
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu>

      2260      2270      2280      2290      2300
      *        *        *        *        *
TAT AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC
ATA TCG TTC GAG TGG CAC CTG TTC TCG TCC ACC GTC GTC CCC TTG CAG
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val>

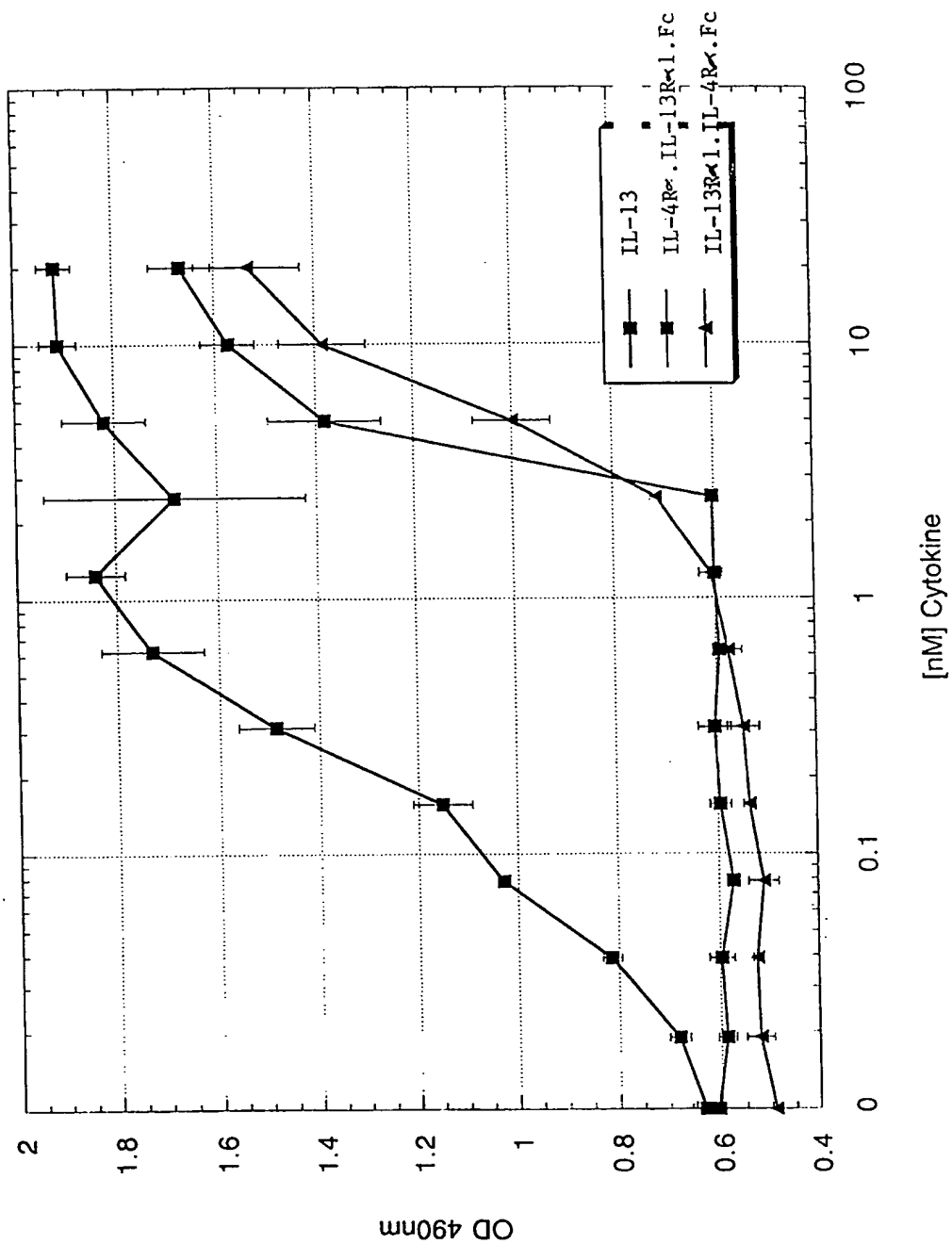
```

Figure 32G

2310			2320			2330			2340			2350			
*	*		*	*		*	*		*	*		*	*		
TTC	TCA	TGC	TCC	GTG	ATG	CAT	GAG	GCT	CTG	CAC	AAC	CAC	TAC	ACG	CAG
AAG	AGT	ACG	AGG	CAC	TAC	GTA	CTC	CGA	GAC	GTG	TTG	GTG	ATG	TGC	GTC
Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln>

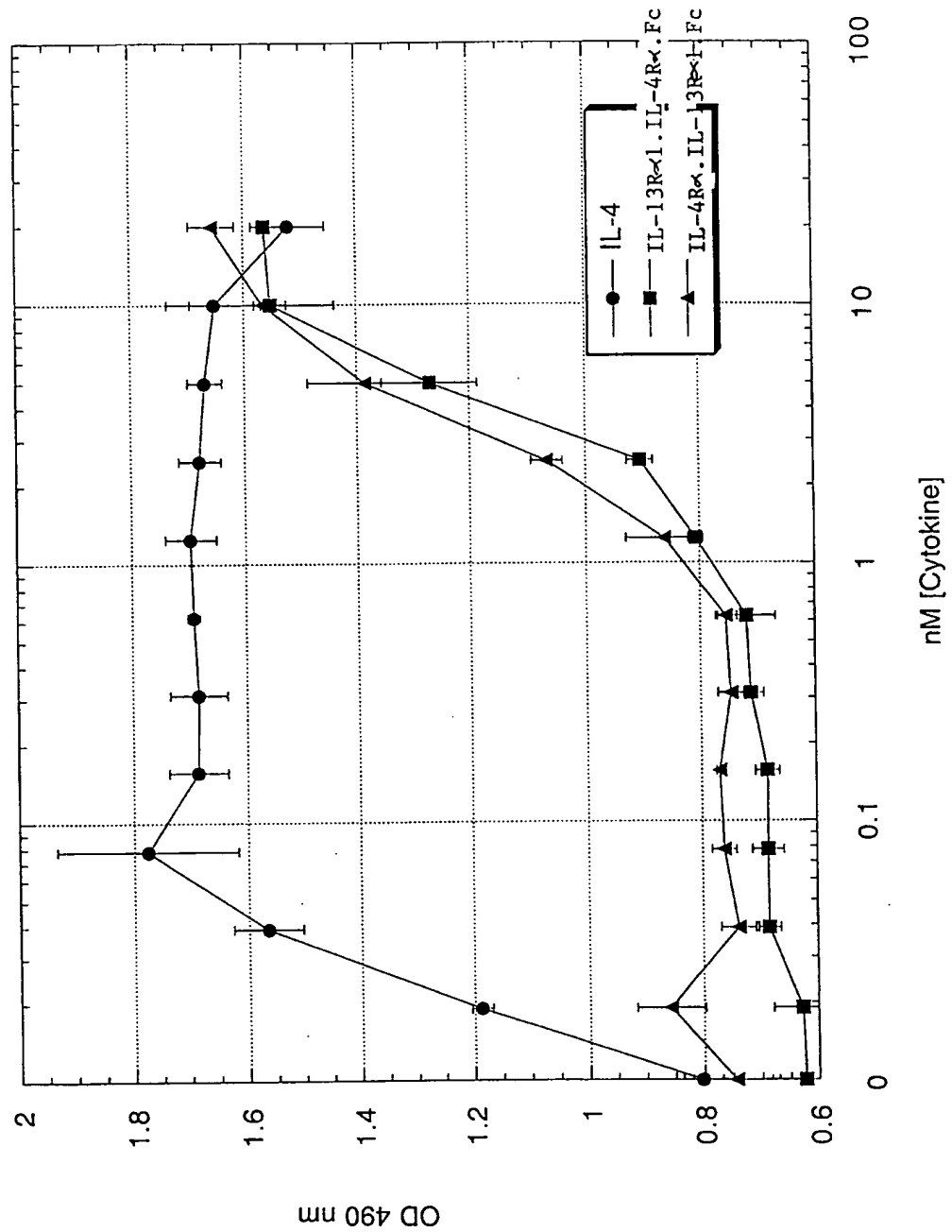
2360			2370			2380			
*	*		*	*		*	*		
AAG	AGC	CTC	TCC	CTG	TCT	CCG	GGT	AAA	TGA
TTC	TCG	GAG	AGG	GAC	AGA	GGC	CCA	TTT	ACT
Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	***>

Figure 33



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Figure 34



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Figure 35

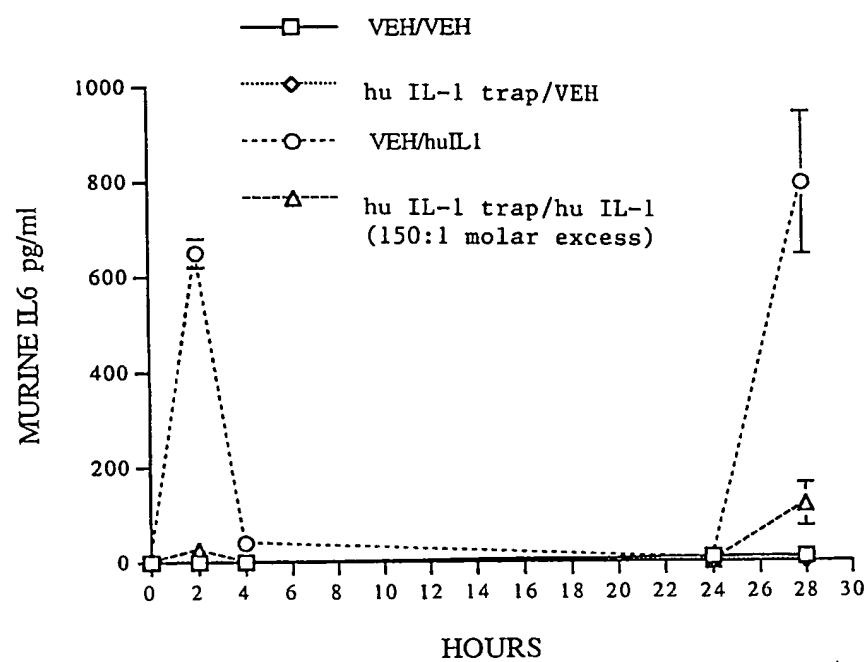


Figure 36A

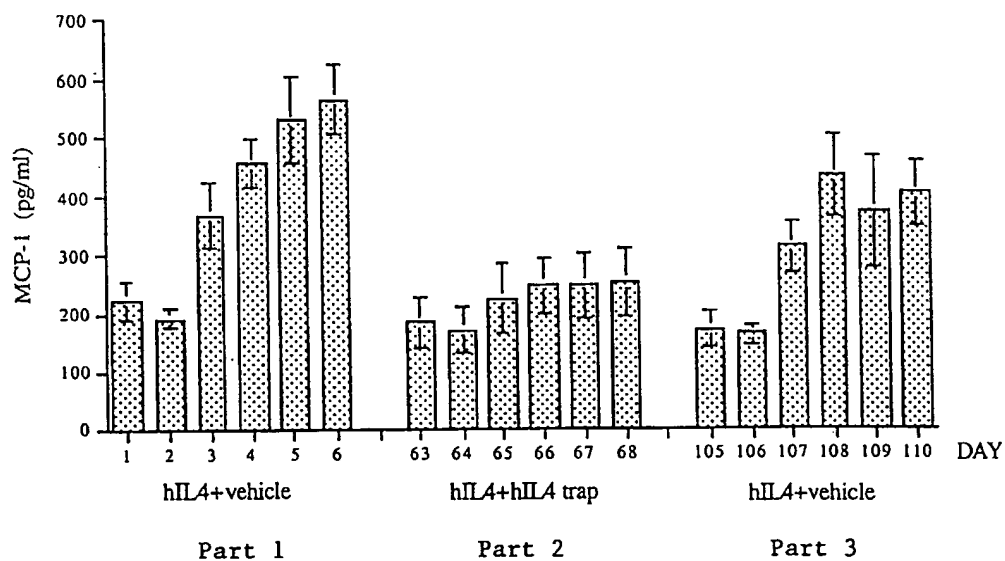


Figure 36B

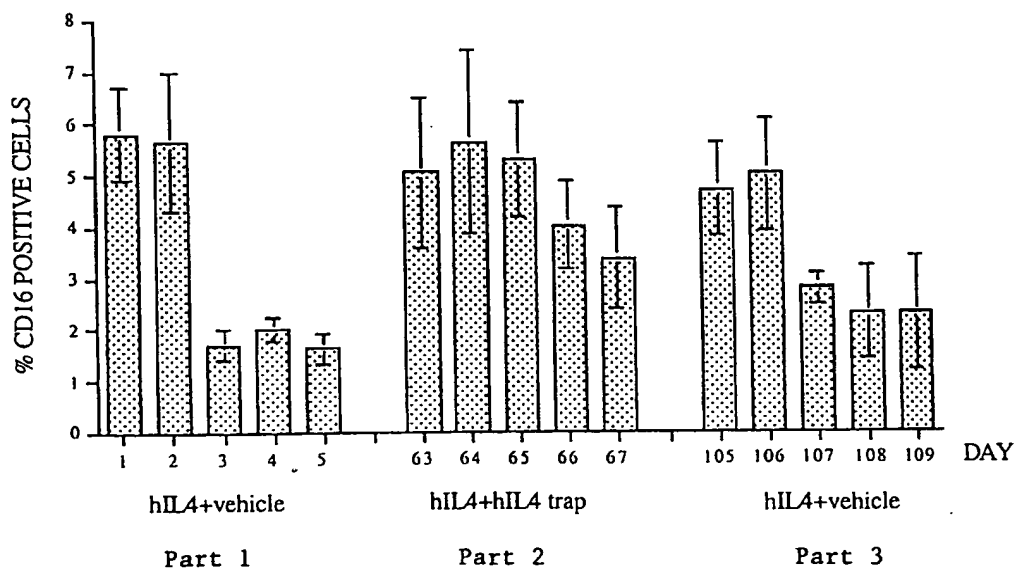
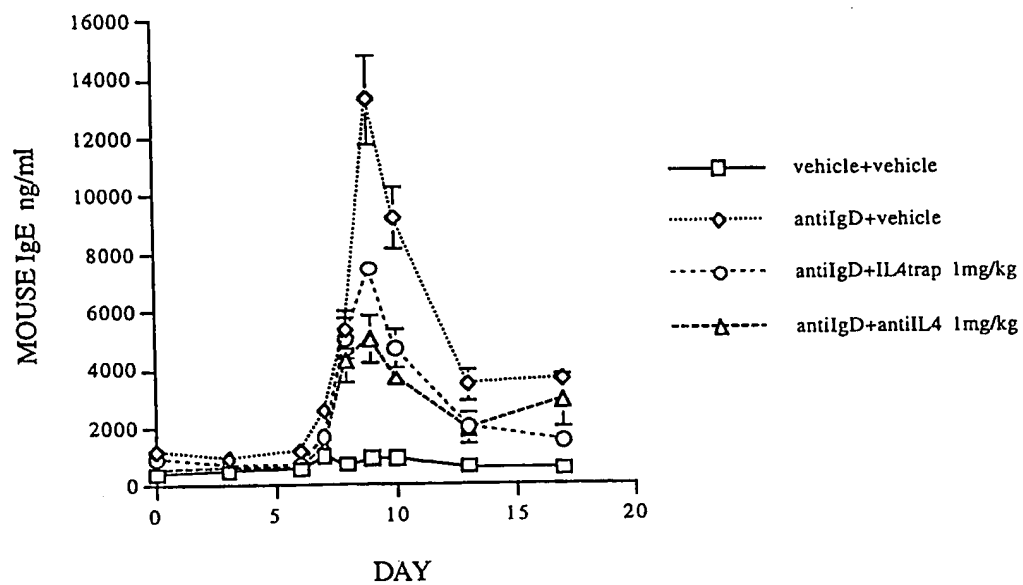


Figure 37





INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N 15/62, C07K 14/715, C12N 15/12	A3	(11) International Publication Number: WO 00/18932 (43) International Publication Date: 6 April 2000 (06.04.00)
(21) International Application Number: PCT/US99/22045 (22) International Filing Date: 22 September 1999 (22.09.99) (30) Priority Data: 60/101,858 25 September 1998 (25.09.98) US 09/313,942 19 May 1999 (19.05.99) US (71) Applicant (for all designated States except US): REGENERON PHARMACEUTICALS, INC. [US/US]; 777 Old Saw Mill River Road, Tarrytown, NY 10591-6707 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): STAHL, Neil [US/US]; RD #10, Kent Shore Drive, Carmel, NY 10512 (US). YAN-COPOULOS, George, D. [US/US]; 1519 Baptist Church Road, Yorktown Heights, NY 10598 (US). (74) Agents: SORRENTINO, Joseph, M.; Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591 (US) et al.	(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> (88) Date of publication of the international search report: 2 November 2000 (02.11.00)	
(54) Title: RECEPTOR BASED ANTAGONISTS AND METHODS OF MAKING AND USING (57) Abstract <p>The present invention provides a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex. It also provides a nucleic acid sequence encoding the fusion polypeptide and methods of making and uses for the fusion polypeptide.</p>		

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/22045

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/62 C07K14/715 C12N15/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 96 23881 A (CELL GENESYS INC) 8 August 1996 (1996-08-08) page 16, line 14 -page 19, line 25 page 47, line 5 -page 48, line 13 page 51, line 12 -page 52, line 3 page 54, line 31 -page 55, line 17 page 58, line 10 -page 59, line 3 page 61, line 33 -page 62, line 20 page 65, line 10 -page 66, line 3 page 69, line 5 - line 25 page 72, line 4 - line 22 page 75, line 10 -page 76, line 3; claims 1,3,4,6,11,12,14,15,17,23,25,26,ETC...; figure 5</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	1-4,9-25



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Date of the actual completion of the international search

13 July 2000

Date of mailing of the international search report

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Smalt, R

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/22045

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 22914 A (HUTCHINSON FRED CANCER RES ;GREENBERG PHILIP D (US); NELSON BRAD H) 13 October 1994 (1994-10-13) the whole document ---	1-4,9-25
X	WO 96 11213 A (AMGEN BOULDER INC) 18 April 1996 (1996-04-18) cited in the application the whole document ---	1-4,9-25
X	WO 97 15669 A (US HEALTH) 1 May 1997 (1997-05-01) page 18, line 27 - line 34; claims 12,16,28 ---	1-4, 10-25
X	WO 93 19163 A (YEDA RES & DEV) 30 September 1993 (1993-09-30) abstract page 7, line 8 -page 8, line 1 page 8, line 31 - line 36 page 17, line 21 - line 24; claims 1,12,16 ---	1,2,4, 10-25
X	WO 95 06737 A (PRENDERGAST KENNETH FRANCIS) 9 March 1995 (1995-03-09) see seq.ID.16 and the claims ---	1-4, 10-25
X	WO 93 19777 A (IMMUNEX CORP) 14 October 1993 (1993-10-14) claims examples 12,13 ---	1-4, 10-25
X	EP 0 835 939 A (HOECHST AG ;GEN HOSPITAL CORP (US)) 15 April 1998 (1998-04-15) claims 8,9,17,21; example 2 ---	1,2,4, 9-25
X	WO 97 31946 A (GENETICS INST) 4 September 1997 (1997-09-04) claims 11,27,28; example 4 ---	1,2,4, 10-25
X	EP 0 533 006 A (HOFFMANN LA ROCHE) 24 March 1993 (1993-03-24) claims 1,3,5,8-10; examples 12,13 ---	1,2,4, 10-25
X	WO 96 35783 A (DARTMOUTH COLLEGE ;CHIRON CORP (US)) 14 November 1996 (1996-11-14) claims 4,5 ---	1,2,4, 10-25

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 99/22045

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SEIPELT I ET AL: "Overexpression, purification, and use of a soluble human interleukin-4 receptor alpha-chain/Ig gamma 1 fusion protein for ligand binding studies. Characterization of ligand binding to soluble IL-4 receptor alpha-chain by surface plasmon resonance measurements and by microtiter-plate-based ELISA with" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS,US,ACADEMIC PRESS INC. ORLANDO, FL, vol. 239, 20 October 1997 (1997-10-20), pages 534-542, XP002105430 ISSN: 0006-291X the whole document ---	1,2,4, 10-18, 23,25
A	WO 96 04389 A (REGENERON PHARMA) 15 February 1996 (1996-02-15) the whole document ---	
A	WO 97 23613 A (CELLTECH THERAPEUTICS LTD ;BEBBINGTON CHRISTOPHER ROBERT (GB); LAW) 3 July 1997 (1997-07-03) page 5, line 32 -page 6, line 30 page 7, line 26 - line 34; claim 26; figures 14-17 ---	
A	WO 98 02558 A (UNIV MEDICINE AND DENTISTRY OF) 22 January 1998 (1998-01-22) page 8, line 25 -page 10, line 5; figures 1,7,10,17 page 25, line 8 -page 28, line 7; claims 1,7-9 ---	
A	WO 95 10611 A (HARVARD COLLEGE) 20 April 1995 (1995-04-20) claims 5,13; example 1 ---	
A	DUSCHL A ET AL: "TRANSMEMBRANE AND INTRACELLULAR SIGNALLING BY INTERLEUKIN - 4: RECEPTOR DIMERIZATION AND BEYOND" EUROPEAN CYTOKINE NETWORK, 1996, XP002061702 figure 4 ---	
A	EP 0 864 585 A (HAYASHIBARA BIOCHEM LAB) 16 September 1998 (1998-09-16) the whole document ---	
A	WO 93 18047 A (LUDWIG INST CANCER RES) 16 September 1993 (1993-09-16) the whole document ---	
	-/--	

INTERNATIONAL SEARCH REPORT

Intern val Application No
PCT/US 99/22045

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>STAHL, N. ET AL.: "Cytokine traps: heteromeric receptor-based protein therapeutics that function as high-affinity blockers of cytokine action." FASEB JOURNAL, vol. 13, no. 7, 23 April 1999 (1999-04-23), pages A1585-Abstr.1457, XP002133324 the whole document</p>	<p>1-4, 10-14</p>
P,X	<p>WO 99 37772 A (BORN TERESA L ;SIMS JOHN E (US); IMMUNEX CORP (US)) 29 July 1999 (1999-07-29) page 3, line 11 - line 26 page 6, line 7 - line 9; examples 1,3,4 page 8, line 22 - line 34 page 10, line 7 - line 15 -----</p>	<p>1-3, 9-18,25</p>

INTERNATIONAL SEARCH REPORT

Int. application No.
PCT/US 99/22045

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
1-4,9-25 (inventions 1 (IL-2 receptor), 3 (IL-4 receptor), 4 (IL-5 receptor), 7 (IL-9 receptor), 9 (IL-13 receptor), 61 (IL-1 receptor), 65 (IL-18 receptor)

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 99/22045

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: claims 1-4,10-25, all partially

Fusion protein, capable of forming a non-functional complex with a cytokine, comprising a specificity-determining component and a signal-transducing component of the IL-2 receptor, and a multimerizing component. Also nucleic acid encoding said fusion protein, (expression) vector comprising said nucleic acid, host cell transformed with said vector, and method for producing said fusion protein using said host.

Inventions 2-66: claims 1-25, all partially,
as far as applicable

As invention 1, but limited to fusion proteins in which the specificity-determining component and the signal-transducing component are derived from the receptors for IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-13, IL-15, GM-CSF, OSM, LIF, cardiotrophin-1, IFN-alpha, IFN-beta, IFN-gamma, B7.1/CD80, B7.2/CD86, TNF-alpha, TNF-beta, LT-beta, CD40-L, FAS-L, CD27-L, CD30-L, 4-1BBL, TGF-beta1, TGF-beta2, TGF-beta3, BMP-2, BMP-3a, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8a, BMP-8b, BMP-9, BMP-10, BMP-11, BMP-15, BMP-16, EBAF, GDF-1, GDF-2, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-12, GDF-14, MIS, activin-1, activin-2, activin-3, activin-4, activin-5, IL-1, IL-10, IL-12, IL-14, IL-18, and MIF, whereby invention 2 relates to fusion protein comprising specificity-determining and signal-transducing components from the IL-3 receptor, invention 3 relates to fusion protein comprising specificity-determining and signal-transducing components from the IL-4 receptor,, invention 65 relates to fusion protein comprising specificity-determining and signal-transducing components from the IL-18 receptor, and invention 66 relates to fusion protein comprising specificity-determining and signal-transducing components from the MIF receptor.

For the sake of conciseness, the first subject matter is explicitly defined, the other subject matters are defined by analogy thereto.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/22045

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9623881 A	08-08-1996	AU 715363 B AU 4861296 A CA 2221634 A EP 0821730 A US 5741899 A US 5837544 A US 6077947 A	03-02-2000 21-08-1996 08-08-1996 04-02-1998 21-04-1998 17-11-1998 20-06-2000
WO 9422914 A	13-10-1994	AU 695869 B AU 6627194 A CA 2160011 A EP 0693084 A JP 8511000 T US 5747292 A	27-08-1998 24-10-1994 13-10-1994 24-01-1996 19-11-1996 05-05-1998
WO 9611213 A	18-04-1996	AU 3830895 A	02-05-1996
WO 9715669 A	01-05-1997	AU 7472496 A	15-05-1997
WO 9319163 A	30-09-1993	AU 3924393 A CA 2132349 A EP 0638119 A JP 7505282 T	21-10-1993 30-09-1993 15-02-1995 15-06-1995
WO 9506737 A	09-03-1995	EP 0716703 A	19-06-1996
WO 9319777 A	14-10-1993	AU 671116 B AU 3970293 A CA 2133326 A EP 0670730 A FI 944516 A JP 7508639 T NO 943617 A NZ 251820 A NZ 280051 A	15-08-1996 08-11-1993 14-10-1993 13-09-1995 22-11-1994 28-09-1995 29-11-1994 26-07-1996 26-07-1996
EP 0835939 A	15-04-1998	AT 169030 T AU 655421 B AU 7935791 A CA 2045869 A DE 59109032 D EP 0464533 A ES 2120949 T GR 3027567 T JP 5247094 A PT 98113 A,B	15-08-1998 22-12-1994 02-01-1992 29-12-1991 03-09-1998 08-01-1992 16-11-1998 30-11-1998 24-09-1993 29-05-1992
WO 9731946 A	04-09-1997	US 5710023 A AU 1980197 A	20-01-1998 16-09-1997
EP 0533006 A	24-03-1993	AU 657788 B AU 2357192 A CA 2078384 A JP 5268967 A NZ 244281 A US 5668256 A US 5712121 A US 5455337 A	23-03-1995 25-03-1993 19-03-1993 19-10-1993 27-04-1995 16-09-1997 27-01-1998 03-10-1995

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/22045

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0533006 A		ZA 9206962 A	13-04-1993
WO 9635783 A	14-11-1996	US 5837816 A	17-11-1998
		AU 5678396 A	29-11-1996
WO 9604389 A	15-02-1996	AU 3239395 A	04-03-1996
		ZA 9506545 A	29-03-1996
WO 9723613 A	03-07-1997	AU 1201997 A	17-07-1997
		CA 2238873 A	03-07-1997
		EP 0870019 A	14-10-1998
		JP 2000502562 T	07-03-2000
WO 9802558 A	22-01-1998	AU 3668897 A	09-02-1998
WO 9510611 A	20-04-1995	AU 701623 B	04-02-1999
		AU 7980694 A	04-05-1995
		CA 2174098 A	20-04-1995
		EP 0726948 A	21-08-1996
		JP 9503673 T	15-04-1997
EP 0864585 A	16-09-1998	AU 4922397 A	17-09-1998
		CA 2219964 A	12-09-1998
		JP 11240898 A	07-09-1999
WO 9318047 A	16-09-1993	AT 172470 T	15-11-1998
		AU 669889 B	27-06-1996
		AU 3779093 A	05-10-1993
		CA 2131719 A	16-09-1993
		DE 69321720 D	26-11-1998
		DE 69321720 T	06-05-1999
		EP 0643719 A	22-03-1995
		ES 2123641 T	16-01-1999
		JP 7504816 T	01-06-1995
		US 5789237 A	04-08-1998
		US 5962269 A	05-10-1999
WO 9937772 A	29-07-1999	AU 2337399 A	09-08-1999
		AU 2466899 A	09-08-1999
		WO 9937773 A	29-07-1999



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(51) International Patent Classification ⁷ : C12N 15/62, C07K 14/715, C12N 15/12	A2	(11) International Publication Number: WO 00/18932 (43) International Publication Date: 6 April 2000 (06.04.00)
(21) International Application Number: PCT/US99/22045 (22) International Filing Date: 22 September 1999 (22.09.99) (30) Priority Data: 60/101,858 25 September 1998 (25.09.98) US 09/313,942 19 May 1999 (19.05.99) US (71) Applicant (for all designated States except US): REGENERON PHARMACEUTICALS, INC. [US/US]; 777 Old Saw Mill River Road, Tarrytown, NY 10591-6707 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): STAHL, Neil [US/US]; RD #10, Kent Shore Drive, Carmel, NY 10512 (US). YAN- COPOULOS, George, D. [US/US]; 1519 Baptist Church Road, Yorktown Heights, NY 10598 (US). (74) Agents: SORRENTINO, Joseph, M.; Regeneron Pharmaceuti- cals, Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591 (US) et al.		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: RECEPTOR BASED ANTAGONISTS AND METHODS OF MAKING AND USING		
(57) Abstract The present invention provides a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex. It also provides a nucleic acid sequence encoding the fusion polypeptide and methods of making and uses for the fusion polypeptide.		

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RECEPTOR BASED ANTAGONISTS AND
METHODS OF MAKING AND USING

5 This application claims priority of U.S. Application No. 09/313,942, filed May 19, 1999, which claims priority of U.S. Provisional Application No. 60/101,858 filed September 25, 1998. Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application.

10

BACKGROUND OF THE INVENTION

Although discovered for varying biological activities, ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), oncostatin M (OSM) and
15 interleukin-6 (IL-6) comprise a defined family of cytokines (referred to herein as the "CNTF family" of cytokines). These cytokines are grouped together because of their distant structural similarities [Bazan, J. Neuron 7: 197-208 (1991); Rose and Bruce, Proc. Natl. Acad. Sci. USA 88: 8641-8645 (1991)], and, perhaps more importantly, because they share " β " signal-
20 transducing receptor components [Baumann, et al., J. Biol. Chem. 265:19853-19862 (1993); Davis, et al., Science 260: 1805-1808 (1993); Gearing et al., Science 255:1434-1437 (1992); Ip et al., Cell 69: 1121-1132 (1992); Stahl, et al., J. Biol. Chem. 268: 7628-7631 (1993); Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. Receptor activation by this family of cytokines results from
25 either homo- or hetero-dimerization of these β components [Davis, et al. Science 260: 1805-1808 (1993), Murakami, et al., Science 260: 1808-1810 (1993); Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. IL-6 receptor activation requires homodimerization of gp130 [Murakami, et al. Science 260: 1808-1810 (1993), Hibi, et al., Cell 63: 1149-1157 (1990)], a protein initially
30 identified as the IL-6 signal transducer [Hibi, et al., Cell 63: 1149-1157 (1990)]. CNTF, LIF and OSM receptor activation results from heterodimerization between gp130 and a second gp130-related protein known as LIFR β [Davis,

et al., Science 260: 1805-1808 (1993)], that was initially identified by its ability to bind LIF [Gearing et al., EMBO J. 10: 2839-2848 (1991)].

In addition to the β components, some of these cytokines also require
5 specificity-determining " α " components that are more limited in their
tissue distribution than the β components, and thus determine the cellular
targets of the particular cytokines [Stahl and Yancopoulos, Cell 74: 587-590
(1993)]. Thus, LIF and OSM are broadly acting factors that may only require
the presence of gp130 and LIFR β on responding cells, while CNTF requires
10 CNTFR α [Stahl and Yancopoulos, Cell 74: 587-590 (1993)] and IL-6 requires
IL-6R α [Kishimoto, et al., Science 258: 593-597 (1992)]. Both CNTFR α
(Davis et al., Science 259:1736-1739 (1993) and IL-6R α [Hibi, et al. Cell
63:1149-1157, Murakami, et al., Science 260:1808-1810 (1990); Taga, et al., Cell
58:573-581 (1989)] can function as soluble proteins, consistent with the
15 notion that they do not interact with intracellular signaling molecules but
that they serve to help their ligands interact with the appropriate signal
transducing β subunits [Stahl and Yancopoulos, Cell 74: 587-590 (1993)].

Additional evidence from other cytokine systems also supports the notion
20 that dimerization provides a common mechanism by which all cytokine
receptors initiate signal transduction. Growth hormone (GH) serves as
perhaps the best example in this regard. Crystallographic studies have
revealed that each GH molecule contains two distinct receptor binding
sites, both of which are recognized by the same binding domain in the
25 receptor, allowing a single molecule of GH to engage two receptor
molecules [de Vos, et al., Science 255: 306-312 (1992)]. Dimerization occurs
sequentially, with site 1 on the GH first binding to one receptor molecule,
followed by the binding of site 2 to a second receptor molecule [Fuh, et al.,
Science 256: 1677-1680 (1992)]. Studies with the erythropoietin (EPO)
30 receptor are also consistent with the importance of dimerization in
receptor activation, as EPO receptors can be constitutively activated by a

single amino acid change that introduces a cysteine residue and results in disulfide-linked homodimers [Watowich, et al., Proc. Natl. Acad. Sci. USA 89:2140-2144 (1992)].

- 5 In addition to homo- or hetero-dimerization of β subunits as the critical step for receptor activation, a second important feature is that formation of the final receptor complex by the CNTF family of cytokines occurs through a mechanism whereby the ligand successively binds to receptor components in an ordered manner [Davis, et al. Science 260:1805-1818 (1993); Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. Thus CNTF first binds to CNTFR α , forming a complex which then binds gp130 to form an intermediate (called here the $\alpha\beta 1$ intermediate) that is not signaling competent because it has only a single β component, before finally recruiting LIFR β to form a heterodimer of β components which then initiates signal transduction. Although a similar intermediate containing IL-6 bound to IL-6R α and a single molecule of gp130 has not been directly isolated, we have postulated that it does exist by analogy to its distant relative, CNTF, as well as the fact that the final active IL-6 receptor complex recruits two gp130 monomers. Altogether, these findings led to a proposal for the structure of a generic cytokine receptor complex (Figure 1) in which each cytokine can have up to 3 receptor binding sites: a site that binds to an optional α specificity-determining component (α site), a site that binds to the first β signal-transducing component ($\beta 1$ site), and a site that binds to the second β signal-transducing component ($\beta 2$ site) [Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. These 3 sites are used in sequential fashion, with the last step in complex formation – resulting in β component dimerization – critical for initiating signal transduction [Davis, et al. Science 260:1805-1818 (1993)]. Knowledge of the details of receptor activation and the existence of the non-functional $\beta 1$ intermediate for CNTF has led to the finding that CNTF is a high affinity

antagonist for IL-6 under certain circumstances, and provides the strategic basis for designing ligand or receptor-based antagonists for the CNTF family of cytokines as detailed below.

- 5 Once cytokine binding induces receptor complex formation, the dimerization of β components activates intracellular tyrosine kinase activity that results in phosphorylation of a wide variety of substrates [Ip, et al. Cell 69:121-1132 (1992)]. This activation of tyrosine kinase appears to be critical for downstream events since inhibitors that block the tyrosine
- 10 phosphorylations also prevent later events such as gene inductions [Ip, et al., Cell 69:121-1132 (1992); Nakajima and Wall, Mol. Cell. Biol. 11:1409-1418 (1991)]. Recently, we have demonstrated that a newly discovered family of non-receptor tyrosine kinases that includes Jak1, Jak2, and Tyk2 (referred to as the Jak/Tyk kinases) [Firmbach-Kraft, et al., Oncogene
- 15 5:1329-1336 (1990); Wilks, et al., Mol. Cell. Biol. 11: 2057-2065 (1991)] and that are involved in signal transduction with other cytokines [Argetsinger, et al., Cell 74:237-244 (1993); Silvennoinen, et al., Proc. Natl. Acad. Sci. USA 90:8429-8433 (1993); Velazquez, et al., Cell 70: 313-322 (1992); Witthuhn, et al., Cell 74:227-236 (1993)], preassociate with the cytoplasmic domains of the
- 20 β subunits gp130 and LIFR β in the absence of ligand, and become tyrosine phosphorylated and activated upon ligand addition [Stahl et al., Science 263:92-95 (1994)]. Therefore these kinases appear to be the most proximal step of intracellular signal transduction activated inside the cell as a result of ligand binding outside of the cell. Assay systems for screening
- 25 collections of small molecules for specific agonist or antagonist activities based on this system are described below.

The CNTF family of cytokines play important roles in a wide variety of physiological processes that provide potential therapeutic applications for

30 both antagonists and agonists.

SUMMARY OF THE INVENTION

An object of the present invention is the production of cytokine antagonists that are useful in the treatment of cytokine-related diseases or disorders.

Another object of the invention is the use of the disclosed cytokine antagonists for the treatment of cytokine-related diseases or disorders. For example, an IL-6 antagonist described herein may be used for the treatment of osteoporosis, the primary and second effects of cancers, including multiple myeloma, or cachexia.

Another object of the invention is the development of screening systems useful for identifying novel agonists and antagonists of cytokine receptors.

Another object of the invention is the development of screening systems useful for identifying small molecules that act as agonists or antagonists of the cytokines.

Another object of the invention is the development of screening systems useful for identifying novel agonists and antagonists of members of the CNTF family of cytokines.

Another object of the invention is the development of screening systems useful for identifying small molecules that act as agonists or antagonists of the CNTF family of cytokines.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1: Ordered binding of receptor components in a model of a generic cytokine receptor. The model indicates that cytokines contain up to 3 receptor binding sites and interact with their receptor components by

binding first the optional α component, followed by binding to $\beta 1$, and then $\beta 2$. The β components for many cytokine receptors interact through membrane proximal regions (shaded boxes) with the Jak/Tyk family of cytoplasmic protein tyrosine kinases. Only upon dimerization of β components is signal transduction initiated, as schematized by the tyrosine phosphorylations (P) of the β components and the Jak/Tyk kinases.

FIGURE 2: CNTF inhibits IL-6 responses in a PC12 cell line (called PC12D) that expresses IL6R α , gp130, CNTFR α , but not LIFR β . Serum-deprived PC12D cells were incubated + IL-6 (50 ng/mL) in the presence or absence of CNTF as indicated. Some plates also received soluble IL6R α (1 mg/mL) or soluble CNTFR α (1 mg/mL) as indicated. Cell lysates were subjected to immunoprecipitation with anti-gp130 and immunoblotted with anti-phosphotyrosine. Tyrosine phosphorylation of gp130 is indicative of IL-6 induced activation of the IL-6 receptor system, which is blocked upon coaddition of CNTF.

FIGURE 3: Scatchard analysis of iodinated CNTF binding on PC12D cells. PC12D cells were incubated with various concentrations of iodinated CNTF in the presence or absence of excess non-radioactive competitor to determine the specific binding. The figure shows a Scatchard plot of the amount of iodinated CNTF specifically bound, and gives data consistent with two binding sites with dissociation constants of 9 pM and 3.4 nM.

FIGURE 4. The amino acid sequence of human gp130-Fc-His₆. Amino acids 1 to 619 are from human gp130 (Hibi et al., Cell 63:1149-1157 (1990). Note that amino acid number 2 has been changed from a Leu to a Val in order to accommodate a Kozak sequence in the coding DNA sequence. The signal peptide of gp130-Fc-His₆ has been italicized (amino acids 1 to 22). The Ser-Gly bridge is shown in bold type (amino acids 620, 621). Amino acids 662 to 853 are from the Fc domain of human IgG1 (Lewis, et

al., J. Immunol. 151:2829-2838 (1993). (+) mark the two cysteines (amino acids number 632 and 635) of the IgG hinge preceding the Fc that form the inter-chain disulfide bridges that link two Fc domains. The hexahistidine tag is shown in bold/italic type (amino acids 854 to 859). (•) shows the position of the STOP codon.

FIGURE 5: The amino acid sequence of human IL-6R α -Fc. Key: Amino acids 1 to 358 are from human IL-6R α (Yamasaki, et al., Science 241:825-828 (1988). Note that amino acid number 2 has been changed from a Leu to a Val in order to accommodate a Kozak sequence in the coding DNA sequence. The signal peptide of IL-6R α -Fc has been italicized (amino acids 1 to 19). The Ala-Gly bridge is shown in bold type (amino acids 359, 360). Amino acids 361 to 592 are from the Fc domain of human IgG1 (Lewis et al., J. Immunol. 151:2829-2838 (1993). (+) mark the two cysteines (amino acids number 371 and 374) of the IgG hinge preceding the Fc that form the inter-chain disulfide bridges that link two Fc domains. (•) shows the position of the STOP codon.

FIGURE 6: The CNTF/IL-6/IL-11 receptor system. The ordered formation of the hexameric signal transducing receptor complex is depicted schematically. The cytokine associates with the R α component to form an obligatory cytokine•R α complex (Kd is about 5 nM). This low affinity complex next associates with the first signal transducing component, marked β 1, to form a high affinity cytokine•R α • β 1 complex (Kd is about 10 pM). In the case of IL-6R α , this component is gp130. This trimeric high affinity complex subsequently associates with another such complex. Formation of this complex results in signal transduction as it involves dimerization of two signal transducing components, marked β 1 and β 2 respectively (adapted from (Ward et al., J. Bio. Chem. 269:23286-23289 (1994); Stahl and Yancopoulos, J. Neurobiology 25:1454-1466 (1994); Stahl and Yancopoulos, Cell 74:587-590 (1993).

- FIGURE 7: Design of heterodimeric receptor-based ligand traps for IL-6. The heterodimeric ligand trap is comprised of two interdisulfide linked proteins, gp130-Fc and IL-6R α -Fc. The gp130-Fc•IL-6R α -Fc complex (upper panel) is shown to mimic the high affinity cytokine•R α • β 1 complex (lower panel). The ligand trap functions as an antagonist by sequestering IL-6 and thus rendering unavailable to interact with the native receptors on IL-6-responsive cells.
- 10 FIGURE 8. Heteromeric immunoglobulin Heavy/Light Chain Receptor Fusions. An example of a heavy/light chain receptor fusion molecule is schematically depicted. The extracellular domain of gp130 is fused to C γ , whereas the extracellular domain of IL-6R α is fused to the constant region of the kappa chain (κ). The inter-chain disulfide bridges are also depicted (S-S).
- 15

- FIGURE 9. Amino acid sequence of gp130-C γ 1. Key: Amino acids 1 to 619 are from human gp130 (Hibi, et al., Cell 63:1149-1157 (1990). Ser-Gly bridge is shown in bold type. Amino acids 662 to 651 are from the constant region of human IgG1 (Lewis et al., J. Immunol. 151:2829-2838 (1993). (*) shows the position of the STOP codon.
- 20

- FIGURE 10: Amino acid sequence of gp130 Δ 3fibro. Key: Amino acids 1 to 330 are from human gp130 (Hibi et al., Cell 63:1149-1157 (1990). Other symbols as described in Figure 9.
- 25

FIGURE 11: Amino acid sequence of J-CH1. Key: The Ser-Gly bridge is shown in bold, the J-peptide is shown in italics, the C H 1 domain is underlined.

FIGURE 12: Amino acid sequence of C γ 4. Key: The Ser-Gly bridge is shown in bold type. Amino acids 2 to 239 comprise the C γ 4 sequence.

FIGURE 13: Amino acid sequence of κ -domain. Key: The Ser-Gly bridge is shown in bold type. Amino acids 2 to 108 comprise the κ domain. The C-terminal cysteine (amino acid 108) is that involved in the disulfide bond of the κ domain with the C μ 1 domain of C γ .

FIGURE 14: Amino acid sequence of λ -domain. Key: The Ser-Gly bridge is shown in bold type. Amino acids 2 to 106 comprise the λ domain (Cheung, et al., J. Virol. 66: 6714-6720 (1992). The C-terminal cysteine (amino acid 106) is that involved in the disulfide bond of the λ domain with the C μ 1 domain of C γ .

FIGURE 15: Amino acid sequence of the soluble IL-6R α domain. Key: Amino acids 1 to 358 comprise the soluble IL-6R α domain (Yamasaki, et al., Science 241:825-828 (1988). The Ala-Gly bridge is shown in bold type.

FIGURE 16: Amino acid sequence of the soluble IL-6R α 313 domain: Key: Amino acids 1 to 313 comprise the truncated IL-6R α domain (IL-6R α 313). The Thr-Gly bridge is shown in bold type.

FIGURE 17: Purification of gp130-C γ 1•IL-6R α - κ . 4% to 12% SDS-PAGE gradient gel run under non-reducing conditions. Proteins were visualized by staining with silver. Lane 1: approximately 100 ng of material purified over Protein A Sepharose (Pharmacia). Lane 2: Molecular size standards (Amersham). Lane 3: The Protein A-purified material shown here after further purification over an IL-6 affinity chromatography step. The positions of the gp130-C γ 1 dimer [(gp130-C γ 1) $_2$], the gp130-C γ 1 dimer

associated with one IL-6R α - κ [(gp130-C γ 1) $_2$ •(IL-6R α - κ) $_1$], and the gp130-C γ 1 dimer associated with two IL-6R α - κ [(gp130-C γ 1) $_2$ •(IL-6R α - κ) $_2$] are shown, as well as the sizes for the molecular size standards in kilodaltons (200, 100, and 46).

5

FIGURE 18: IL-6 dissociates slowly from the ligand trap. The dissociation rate of IL-6 from a heavy/light chain receptor-based ligand trap (gp130-C γ 1•IL-6R α - κ) was compared to that obtained with the neutralizing monoclonal antibody B-E8 (BE8 MAb).

10

FIGURE 19: IL-6 can induce multimerization of the ligand trap. (A) Two different ligand traps are depicted schematically and listed according to their ability to bind protein A. gp130-Fc•IL-6R α -Fc (GF6F) binds protein A via its Fc-domains, whereas gp130-CH1•IL-6R α - κ (G16K) does not bind to protein A. (B) Anti-kappa western blotting of proteins precipitated with Protein A-Sepharose from mixtures of GF6F \pm IL-6, G16K \pm IL-6, or GF6F plus G16K \pm IL-6, as marked.

15

FIGURE 20: Inhibition of IL-6-dependent XG-1 cell proliferation. XG-1 cells [Zhang, et al., Blood 83:3654-3663 (1994)] were prepared for a proliferation assay by starving the cells from IL-6 for 5 hours. Assays were set up in 96-well tissue culture dishes in RPMI + 10% fetal calf serum + penicillin/streptomycin + 0.050 nM 2-mercaptoethanol + glutamine. 0.1 ml of that media was used per well. Cells were suspended at a density of 250,000 per ml at the start of the assay. 72 hours post addition of IL-6 \pm ligands traps or antibodies, an MTT assay was performed as described (Panayotatos et al. Biochemistry 33:5813-5818 (1994). The different ligand traps utilized are listed.

20

25

FIGURES 21A-21D: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 424 which is capable of binding the cytokine IL-4 to form a nonfunctional complex.

- 5 FIGURES 22A-22D: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 603 which is capable of binding the cytokine IL-4 to form a nonfunctional complex.

- 10 FIGURES 23A-23D: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 622 which is capable of binding the cytokine IL-4 to form a nonfunctional complex.

- 15 FIGURE 24A-24F: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 412 which is capable of binding the cytokine IL-6 to form a nonfunctional complex.

- 20 FIGURE 25A-25F: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 616 which is capable of binding the cytokine IL-6 to form a nonfunctional complex.

- FIGURE 26A-26E: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 569 which is capable of binding the cytokine IL-1 to form a nonfunctional complex.

- 25 FIGURE 27: Shows that an IL-4 trap designated 4SC375, which is a fusion polypeptide of IL-2R γ -scb-IL4R α -Fc Δ C1, is several orders of magnitude better as an IL-4 antagonist than IL4R α Fc Δ C1 alone in the TF1 cell bioassay.

- 30 FIGURE 28: Shows that an IL-4 trap designated 4SC375 displays antagonistic activity in the TF1 cell bioassay equivalent to an IL-4 trap designated 4SC424 (described in Figs. 21A-21D) which is a fusion

polypeptide of IL-2R γ -IL4R α -Fc Δ C1 having the IL-2R γ component flush with the IL-4R α component.

FIGURE 29: Shows that the IL6 trap (6SC412 IL6R-scb-gpx-Fc Δ C1) described
5 in Figs. 24A-24F is a better antagonist of IL-6 in the XG1 bioassay than the neutralizing monoclonal antibody to human IL-6 - BE8.

FIGURE 30: Shows that the trap 1SC569 (described in Figs. 26A-26E) is able
10 to antagonize the effects of IL-1 and block the IL-6 production from MRC 5 cells upon treatment with IL-1.

FIGURE 31A-31G: The nucleotide and encoded amino acid sequence of the IL-4R α .IL-13R α 1.Fc single chain trap construct is set forth.

15 FIGURE 32A-32G: The nucleotide and encoded amino acid sequence of the IL-13R α 1.IL-4R α .Fc single chain trap construct is set forth.

FIGURE 33: Blocking of IL-13 by IL-4R α .IL-13R α 1.Fc and IL-13R α 1.IL-4R α .Fc. Addition of either IL-4R α .IL-13R α 1.Fc or IL-13R α 1.IL-4R α .Fc trap
20 at a concentration of 10nM blocks IL-13-induced growth up to ~2nM. At an IL-13 concentration of ~4-5 nM the growth of TF1 cells is inhibited by 50%.

FIGURE 34: Blocking of IL-4 by IL-4R α .IL-13R α 1.Fc and IL-13R α 1.IL-4R α .Fc. Addition of either IL-4R α .IL-13R α 1.Fc or IL-13R α 1.IL-4R α .Fc at a
25 concentration of 10nM blocks IL-4-induced growth up to ~1nM. At an IL-4 concentration of ~3-4 nM the growth of TF1 cells is inhibited by 50%.

FIGURE 35: Human IL-1 trap blocks the in vivo effects of exogenously administered huIL-1. BALB/c mice were given subcutaneous injection of
30 huIL-1 (0.3 μ g/kg) at time 0. Twenty-four hours prior to huIL-1 injection, the animals were pre-treated with either vehicle or 150-fold molar excess

of huIL-1 trap. Two hours prior to sacrifice (26 hrs), the mice were re-challenged with a second injection of huIL-1 (0.3 µg/kg, s.c.). Blood samples were collected at various time points and sera were assayed for IL-1 levels (expressed as mean +/- SEM; n=5 per group).

5

FIGURE 36A & FIGURE 36B: Human IL-4 trap antagonizes the effects of human IL-4 in monkeys. Figure 36A: Cynomolgus monkeys were treated in three parts as indicated. Human IL-4 (25 µg/kg) was injected subcutaneously twice daily for 4 days and human IL-4 trap (8 mg/ml) and vehicle were given intravenously daily for 5 days, beginning 1 day prior to human IL-4 administration. Plasma was collected daily and assayed for MCP-1 levels. Results were expressed as mean +/- SEM; n=4. (ANOVA p<0.0007; Tukey-Kramer: Part 2 vs. Part 1, p,0.05; Part 2 vs. Part 3, p,0.05; Part 1 vs. Part 3, not significant.) Figure 36B: Cynomolgus monkeys were treated in three parts as indicated. Human IL-4 (25 µg/kg) was injected subcutaneously twice daily for 4 days and human IL-4 trap (8 mg/ml) and vehicle were given intravenously daily for 5 days, beginning 1 day prior to human IL-4 administration. Whole blood was collected daily for flow cytometry analysis for CD16. Results were expressed as mean +/- SEM; n=4. (ANOVA p<0.042; Tukey-Kramer: Part 2 vs. Part 1, p<0.05; Part 2 vs. Part 3 and Part 1 vs. Part 3, not significant.)

FIGURE 37: Murine IL-4 trap partially prevented IL-4-mediated IgE increase in mice. BALB/C mice injected with anti-mouse IgD (100µl/mouse, s.c.) were randomly divided into 3 groups, each received (on days 3-5) either vehicle, murine IL-4 trap (1 mg/kg, s.c.), or a monoclonal antibody to mouse IL-4 (1 mg/kg, s.c.). Sera were collected at various time points and assayed for IgE levels. Results were expressed as mean+/-SEM (n=5 per group). (ANOVA p=0.0002; Tukey-Kramer: vehicle vs. IL-4 trap, p<0.01; vehicle vs. IL-4 antibody, p<0.001; IL-4 trap vs. IL-4 antibody, not significant).

DETAILED DESCRIPTION OF THE INVENTION

- The present invention provides an isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising:
- a) a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the specificity determining component of the cytokine's receptor;
 - b) a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the signal transducing component of the cytokine's receptor; and
 - c) a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a multimerizing component.

By "cytokine binding portion" what is meant is the minimal portion of the extracellular domain necessary to bind the cytokine. It is accepted by those of skill in the art that a defining characteristic of a cytokine receptor is the presence of the two fibronectin-like domains that contain canonical cysteines and of the WSXWS box (Bazan, J.F., 1990, PNAS 87: 6934-6938). Sequences encoding the extracellular domains of the binding component of the cytokine's receptor and of the signal transducing component of the cytokine's receptor may also be used to create the fusion polypeptide of the invention. Similarly, longer sequences encoding larger portions of the components of the cytokine's receptor may be used. However, it is contemplated that fragments smaller than the extracellular domain will function to bind the cytokine and therefore, the invention contemplates fusion polypeptides comprising the minimal portion of the extracellular domain necessary to bind the cytokine as the cytokine binding portion.

The invention comprises a "specificity determining component" of a cytokine's receptor and a "signal transducing component" of the cytokine's receptor. Regardless of the nomenclature used to designate a particular component or subunit of a cytokine receptor, one skilled in the art would
5 recognize which component or subunit of a receptor is responsible for determining the cellular target of the cytokine, and thus would know which component constitutes the "specificity determining component."

Similarly, regardless of the nomenclature used, one of skill in the art
10 would know which component or subunit of a receptor would constitute the "signal transducing component." As used herein, the "signal transducing component" is a component of the native receptor which is not the specificity determining component and which does not bind or weakly binds the cytokine in the absence of the specificity determining
15 component. In the native receptor, the "signal transducing component" may participate in signaling.

For example, while some cytokine receptors have components designated α and β , the IL-4 receptor has a signal transducing component referred to
20 as IL-2R γ . However, regardless of what name is associated with that component, one skilled in the art would know which component of the IL-4 receptor is the signal transducing component. Thus to practice the present invention and create a high affinity trap for IL-4, one of skill in the art would create an isolated nucleic acid comprising a nucleotide sequence
25 encoding a first fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the specificity determining component of the IL-4 receptor (IL-4R α); a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the
30 extracellular domain of the signal transducing component of the IL-4 receptor (IL-2R γ); and a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a

multimerizing component (for example, an Fc domain of IgG) to create a high affinity trap for IL-4.

5 Some further examples of the receptor components that may be used to prepare cytokine antagonists according to the invention are set forth in Table 1. The Table 1 sets forth, by way of example but not by way of limitation, some of the varied nomenclature used in the scientific literature to describe those components which function as specificity determining components and those which function as signal transducing
10 components of certain cytokine receptors.

TABLE 1

<u>Cytokine</u>	<u>Specificity determining Component</u>	<u>Signal transducing Component</u>
Interleukin-1 (IL-1)	Type I IL-1R (ref. 8) Type II IL-1R (ref. 8) IL-1RI (ref. 11) IL-1RII (ref. 11)	IL-1R AcP (refs. 8, 11)
Interleukin-2 (IL-2)	α -subunit (ref. 2) α -chain (ref. 3) IL-2R α (ref. 1)	β -chain (ref. 3) β -subunit (ref. 2) γ -chain (ref. 3) IL-2R β (refs. 1, 10) IL-2R γ (refs. 1, 10)
Interleukin-3 (IL-3)	IL-3R α (ref. 1) α -subunit (ref. 2) α -receptor component (ref. 5)	β_c (ref. 1) β -subunit (ref. 2) β -chain (ref. 3) β -receptor component (ref. 5)
Interleukin-4 (IL-4)	IL-4R (ref. 1)	γ -chain (ref. 3) IL-2R γ (ref. 1)
Interleukin-5 (IL-5)	IL-5R α (ref. 1) α -subunit (ref. 2) α -receptor component (ref. 5)	β_c (ref. 1) β -subunit (ref. 2) β -chain (ref. 3) β -receptor component (ref. 5)

TABLE 1 (CONT'D)

<u>Cytokine</u>	<u>Specificity determining Component</u>	<u>Signal transducing Component</u>
Granulocyte macrophage-colony stimulating factor (GM-CSF)	α -receptor component (ref. 5) α -subunit (ref. 2) GMR α (refs. 1, 2)	β -receptor component (ref. 5) β -subunit (ref. 2) β -chain (ref. 3) β_c (ref. 1) GMR β (refs. 1, 2)
Leukemia inhibitory factor (LIF)	LIFBP (ref. 1) α -receptor component (ref. 5)	gp130 (refs. 1, 3) β -receptor component (ref. 5)
Interleukin-11 (IL-11)	α -chain (ref. 4) NR1 (ref. 4)	gp130 (ref. 4)
Interleukin-15 (IL-15)	IL-15R α (ref. 10)	IL-2R β (ref. 10) IL-2R γ (ref. 10)
Interferon- γ (IFN γ)	IFN- γ R (ref. 7) IFN- γ R1 (ref. 7)	AF-1 (ref. 7) IFN- γ R2 (ref. 7)
TGF β	Type II (refs. 6, 9)	Type I (refs. 6, 9)

Only a few of the multitude of references are cited in Table 1, and they are set forth as follows:

1. Sato and Miyajima, Current Opinions in Cell Biology 6: 174-179
5 (1994) - See page 176, lines 9-16;
2. Miyajima, et al., Annual Review of Immunology 10: 295-331 (1992) -
See page 295, line 4 to page 296, line 1; page 305, last paragraph;
3. Kondo, et al, Science 262: 1874-1877 (1993) - See page 1874, cols. 1 & 2;
4. Hilton, et al, EMBO Journal 13: 4765-4775 (1994) - See page 4766, col.
10 1, lines 20-24;
5. Stahl and Yancopoulos, Cell 74: 587-590 (1993) - See page 587,
column 2, lines 15-22;
6. Bassing, et al, Journal of Biological Chemistry 269: 14861-14864 (1994)
- See page 14861, col. 2, lines 1-9 and 21-28;
- 15 7. Kotenko, et al, Journal of Biological Science 270: 20915-20921 (1995) -
See page 20915, lines 1-5 of the abstract;
8. Greenfeder, et al., Journal of Biological Chemistry 270: 13757-13765
(1995) - See page 13757, col. 1, line 6 to col. 2, line 3 and col. 2, lines 10-12;
page 13764, col. 2, last 3 lines and page 13765, col. 1, lines 1-7;
- 20 9. Lebrun and Vale, Molecular Cell Biology 17: 1682-1691 (1997) - See
page 1682, Abstract lines 2-6;
10. Kennedy and Park, Journal of Clinical Immunology 16: 134-143
(1996) - See page 134, lines 1-7 of the abstract; page 136, col 2., lines 1-5;
11. Wesche, et al., Journal of Biological Chemistry 272: 7727-7731 (1997)
25 See page 7731, lines 20-26.

Kotenko, et al. recently identified the IL-10R2 (IL-10R β) chain which is reported to serve as an accessory chain that is essential for the active IL-10 receptor complex and for initiating IL-10 induced signal transduction events (S.V. Kotenko, et al., The EMBO Journal, 1997, Vol. 16: 5894-5903).
30 Additional cytokines and their receptors are described in Appendix II, page A:9 of Immunobiology, The Immune System In Health and Disease, 2nd

Edition, by Charles A. Janeway, Jr. and Paul Travers, published by Current Biology Ltd./Garland Publishing Inc., copyright 1996.

In preparing the nucleic acid sequence encoding the fusion polypeptide of
5 the invention, the first, second, and third components of the fusion
polypeptide are encoded in a single strand of nucleotides which, when
expressed by a host vector system, produces a monomeric species of the
fusion polypeptide. The monomers thus expressed then multimerize due
to the interactions between the multimerizing components (the third
10 fusion polypeptide components). Producing the fusion polypeptides in
this manner avoids the need for purification of heterodimeric mixtures
that would result if the first and second components were produced as
separate molecules and then multimerized. For example, U.S. Patent No.
5,470,952 issued November 28, 1995 describes the production of
15 heterodimeric proteins that function as CNTF or IL-6 antagonists. The
heterodimers are purified from cell lines cotransfected with the
appropriate alpha (α) and beta (β) components. Heterodimers are then
separated from homodimers using methods such as passive elution from
preparative, nondenaturing polyacrylamide gels or by using high pressure
20 cation exchange chromatography. The need for this purification step is
avoided by the methods of the present invention.

In addition, PCT International Application WO 96/11213 published 18
April 1996 entitled Dimeric IL-4 Inhibitors states that the applicant has
25 prepared homodimers in which two IL-4 receptors are bound by a
polymeric spacer and has prepared heterodimers in which an IL-4 receptor
is linked by a polymeric spacer to an IL-2 receptor gamma chain. The
polymeric spacer described is polyethylene glycol (PEG). The two receptor
components, IL-4R and IL-2Rgamma are separately expressed and purified.
30 Pegylated homodimers and heterodimers are then produced by joining the
components together using bi-functional PEG reagents. It is an advantage

of the present invention that it avoids the need for such time consuming and costly purification and pegylation steps.

In one embodiment of the invention, the nucleotide sequence encoding
5 the first component is upstream of the nucleotide sequence encoding the
second component. In another embodiment of the invention, the
nucleotide sequence encoding the first component is downstream of the
nucleotide sequence encoding the second component. Further
embodiments of the invention may be prepared in which the order of the
10 first, second and third fusion polypeptide components are rearranged. For
example, if the nucleotide sequence encoding the first component is
designated 1, the nucleotide sequence encoding the second component is
designated 2, and the nucleotide sequence of the third component is
designated 3, then the order of the components in the isolated nucleic acid
15 of the invention as read from 5' to 3' may be any of the following six
combinations: 1,2,3; 1,3,2; 2,1,3; 2,3,1; 3,1,2; or 3,2,1.

In further embodiments of the invention, the cytokine bound by the
fusion polypeptide may be a member of the hematopoietin family of
20 cytokines selected from the group consisting of interleukin-2, interleukin-
3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-9,
interleukin-11, interleukin-13, interleukin-15, granulocyte macrophage
colony stimulating factor, oncostatin M, leukemia inhibitory factor, and
cardiotrophin-1.

25

In additional embodiments of the invention, the cytokine bound by the
fusion polypeptide may be a member of the interferon family of cytokines
selected from the group consisting of IFN-gamma, IFN-alpha, and IFN-
beta.

30

In additional embodiments of the invention, the cytokine bound by the
fusion polypeptide may be a member of the immunoglobulin superfamily

of cytokines selected from the group consisting of B7.1 (CD80) and B7.2 (B70).

5 In still further embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the TNF family of cytokines selected from the group consisting of TNF-alpha, TNF-beta, LT-beta, CD40 ligand, Fas ligand, CD 27 ligand, CD 30 ligand, and 4-1BBL.

10 In additional embodiments of the invention, the cytokine bound by the fusion polypeptide may be a cytokine selected from the group consisting of interleukin-1, interleukin-10, interleukin-12, interleukin-14, interleukin-18, and MIF.

15 Because specificity determination and signal transduction occurs by a similar mechanism in the TGF- β /BMP family of cytokines (See D. Kingsley, *Genes & Development*, 1994, 8: 133-146; J. Wrana, *Miner Electrolyte Metab*, 24: 120-130 (1998); R. Derynck and X. Feng, *Biochimica et Biophysica Acta* 1333 (1997) F105-F150; and J. Massague and F. Weis-Garcia, "Serine/threonine Kinase Receptors: Mediators of Transforming Growth
20 Factor Beta Family Signals" *In Cancer Surveys*, Vol. 27: Cell Signaling, 1996, Imperial Cancer Research Fund) the present invention may be used to produce high affinity antagonists for cytokines that are members of the TGF- β /BMP family.

25 Therefore, in additional embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the TGF- β /BMP family selected from the group consisting of TGF- β 1, TGF- β 2, TGF- β 3, BMP-2, BMP-3a, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8a, BMP-8b, BMP-9, BMP-10, BMP-11, BMP-15, BMP-16, endometrial bleeding
30 associated factor (EBAF), growth differentiation factor-1 (GDF-1), GDF-2, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-12, GDF-14, mullerian

inhibiting substance (MIS), activin-1, activin-2, activin-3, activin-4, and activin-5.

In alternative embodiments of the invention, the specificity determining
5 component, the signal transducing component, or both, may be substituted
for by a single chain Fv. A single chain Fv (scFv) is a truncated Fab having
only the V region of a heavy chain linked by a stretch of synthetic peptide
to a V region of a light chain. See, for example, US Patent Nos. 5,565,332;
5,733,743; 5,837,242; 5,858,657; and 5,871,907 assigned to Cambridge
10 Antibody Technology Limited incorporated by reference herein. Thus the
present invention contemplates, for example, an isolated nucleic acid
molecule encoding a fusion polypeptide capable of binding a cytokine to
form a nonfunctional complex comprising a nucleotide sequence encoding
a first fusion polypeptide component comprising the amino acid sequence
15 of the cytokine binding portion of the extracellular domain of the
specificity determining component of the cytokine's receptor; a nucleotide
sequence encoding a second fusion polypeptide component comprising the
amino acid sequence of an scFv capable of binding the cytokine at a site
different from the site at which the cytokine binding portion of the
20 extracellular domain of the specificity determining component of the
cytokine's receptor binds; and a nucleotide sequence encoding a third
fusion polypeptide component comprising the amino acid sequence of a
multimerizing component. Alternatively, the specificity determining
component may be substituted for by a scFv that binds to a site on the
25 cytokine different from the site at which the signal transducing
component binds. Thus the invention contemplates an isolated nucleic
acid molecule encoding a fusion polypeptide capable of binding a cytokine
to form a nonfunctional complex comprising a nucleotide sequence
encoding a first fusion polypeptide component comprising the amino acid
30 sequence of a scFv that binds to a site on the cytokine different from the
site at which the cytokine binding portion of the extracellular domain of
the signal transducing component of the cytokine's receptor binds; a
nucleotide sequence encoding a second fusion polypeptide component

comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the signal transducing component of the cytokine's receptor; and a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a
5 multimerizing component.

In another embodiment, the invention contemplates an isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising a nucleotide sequence
10 encoding a first fusion polypeptide component comprising the amino acid sequence of a first scFv that binds to a site on the cytokine; a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence a second scFv that binds to a site on the cytokine different from the site at which the first scFv binds; and a nucleotide
15 sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a multimerizing component.

In all of the above described embodiments comprising scFv's, the invention also contemplates embodiments in which the nucleotide
20 sequence encoding the first component is upstream of the nucleotide sequence encoding the second component; embodiments in which the nucleotide sequence encoding the first component is downstream of the nucleotide sequence encoding the second component; and further embodiments of the invention in which the order of the first, second and
25 third fusion polypeptide components is rearranged. For example, if the nucleotide sequence encoding the first component is designated 1, the nucleotide sequence encoding the second component is designated 2, and the nucleotide sequence of the third component is designated 3, then the order of the components in the isolated nucleic acid of the invention as
30 read from 5' to 3' may be any of the following six combinations: 1,2,3; 1,3,2; 2,1,3; 2,3,1; 3,1,2; or 3,2,1.

In preferred embodiments of the invention, the multimerizing component comprises an immunoglobulin derived domain. More specifically, the immunoglobulin derived domain may be selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG. In another embodiment, the multimerizing component may be an Fc domain from which the first five amino acids (including a cysteine) have been removed to produce a multimerizing component referred to as Fc(Δ C1). Alternatively, the multimerizing component may be an Fc domain in which a cysteine within the first five amino acids has been substituted for by another amino acid such as, for example, serine or alanine.

The present invention also provides for fusion polypeptides encoded by the isolated nucleic acid molecules of the invention. Preferably, the fusion polypeptides are in multimeric form, due to the function of the third multimerizing component. In a preferred embodiment, the multimer is a dimer. Suitable multimerizing components are sequences encoding an immunoglobulin heavy chain hinge region (Takahashi et al., 1982, Cell 29:671-679); immunoglobulin gene sequences, and portions thereof. In a preferred embodiment of the invention, immunoglobulin gene sequences, especially one encoding the Fc domain, are used to encode the third multimerizing component.

The present invention also contemplates a vector which comprises the nucleic acid molecule of the invention as described herein.

Also provided is an expression vector comprising a nucleic acid molecule of the invention as described herein, wherein the nucleic acid molecule is operatively linked to an expression control sequence. Also provided is a host-vector system for the production of a fusion polypeptide which comprises the expression vector of the invention which has been introduced into a host cell suitable for expression of the fusion

polypeptide. The suitable host cell may be a bacterial cell such as E. coli, a yeast cell, such as Pichia pastoris, an insect cell, such as Spodoptera frugiperda, or a mammalian cell, such as a COS, CHO, 293, BHK or NS0 cell.

5

The present invention also provides for methods of producing the fusion polypeptides of the invention by growing cells of the host-vector systems described herein, under conditions permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.

10

The present invention provides novel antagonists which are based on receptor components that are shared by cytokines such as the CNTF family of cytokines.

15 The invention described herein contemplates the production of antagonists to any cytokine that utilizes an α specificity determining component which, when combined with the cytokine, binds to a first β signal transducing component to form a nonfunctional intermediate which then binds to a second β signal transducing component causing β -
20 receptor dimerization and consequent signal transduction. According to the invention, the soluble α specificity determining component of the receptor ($sR\alpha$) and the extracellular domain of the first β signal transducing component of the cytokine receptor ($\beta 1$) are combined to form heterodimers ($sR\alpha:\beta 1$) that act as antagonists to the cytokine by binding the
25 cytokine to form a nonfunctional complex.

As described in Example 1, CNTF and IL-6 share the $\beta 1$ receptor component gp130. The fact that CNTF forms an intermediate with CNTFR α and gp130 can be demonstrated (Example 1) in cells lacking
30 LIFR β , where the complex of CNTF and CNTFR α binds gp130, and

- prevents homodimerization of gp130 by IL-6 and IL-6R α , thereby blocking signal transduction. These studies provide the basis for the development of the IL-6 antagonists described herein, as they show that if, in the presence of a ligand, a nonfunctional intermediate complex, consisting of the ligand, its α receptor component and its β 1 receptor component, can be formed, it will effectively block the action of the ligand. Other cytokines may use other β 1 receptor components, such as LIFR β , which may also be used to produce antagonists according to the present invention.
- Thus for example, in one embodiment of the invention, effective antagonists of IL-6 or CNTF consist of heterodimers of the extracellular domains of the α specificity determining components of their receptors (sIL-6R α and sCNTFR α , respectively) and the extracellular domain of gp130. The resultant heterodimers, which are referred to hereinafter as sIL-6R α : β 1 and sCNTFR α : β 1, respectively, function as high-affinity traps for IL-6 or CNTF, respectively, thus rendering the cytokine inaccessible to form a signal transducing complex with the native membrane-bound forms of their receptors.
- Although soluble ligand binding domains from the extracellular portion of receptors have proven to be somewhat effective as traps for their ligands and thus act as antagonists [Bargetzi, et al., Cancer Res. 53:4010-4013 (1993); , et al., Proc. Natl. Acad. Sci. USA 89: 8616-8620 (1992); Mohler, et al., J. Immunol. 151: 1548-1561 (1993); Narazaki, et al., Blood 82: 1120-1126 (1993)], the IL-6 and CNTF receptors are unusual in that the α receptor components constitute ligand binding domains that, in concert with their ligands, function effectively in soluble form as receptor agonists [Davis, et al. Science 259:1736-1739 (1993); Taga, et al., Cell 58: 573-581 (1989)]. The sR α : β 1 heterodimers prepared according to the present invention provide effective traps for their ligands, binding these ligands with affinities in the picomolar range (based on binding studies for CNTF to PC12D cells)

without creating functional intermediates. The technology described herein may be applied to develop a cytokine trap for any cytokine that utilizes an α -component that confers specificity, as well as a β component which, when bound to the α -specificity component, has a higher affinity for the cytokine than either component alone. Accordingly, antagonists according to the invention include antagonists of interleukins 1 through 5 [IL-1, Greenfeder, et al. J. Biol. Chem. 270:13757-13765 (1995); Guo, et al. J. Biol. Chem. 270:27562-27568 (1995)], IL-2; [Taniguchi, et al. European Patent Nos. 0386289-A and 0386304-A (1990); Takeshita, et al. Science 257:379-382 (1992)]; IL-3; [Kitamura, et al. Cell 66:1165-1174 (1991)], IL-4; [Idzerda, et al. J. Exp. Med. 171:861-873 (1990)], IL-5; [Taverneir, et al. Cell 66:1175-1184 (1991)], IL-11 [(Cherel, et al. Direct Submission to EMBL/GenBank/DDBJ databases; accession No. Z38102)], interleukin 15 [IL-15; Hemar, et al. J. Cell Biol. 1295:55-64 (1995); Taniguchi, et al. European Patent Nos. 0386289-A and 0386304-A (1990); Takeshita, et al. Science 257:379-382 (1992)], granulocyte-macrophage colony stimulating factor [GM-CSF; Hayashida, et al. Proc. Natl. Acad. Sci. U.S.A. 97:9655-9659 (1990)], LIF, gamma interferon [IFN γ ; Aguet, et al. Cell 55:273-280 (1988); Soh, et al. Cell 76:793-802 (1994)], and transforming growth factor beta [TGF β ; Inagaki, et al. Proc. Natl. Acad. Sci. USA 90:5359-5363 (1993)].

The α and β receptor extracellular domains may be prepared using methods known to those skilled in the art. The CNTFR α receptor has been cloned, sequenced and expressed [Davis, et al. (1991) Science 253:59-63 which is incorporated by reference in its entirety herein]. The cloning of LIFR β and gp130 are described in Gearing et al. in EMBO J. 10:2839-2848 (1991), Hibi, et al. Cell 63:1149-1157 (1990) and in published PCT application WO 93/10151 published May 27, 1993, all of which are incorporated by reference in their entirety herein.

30

The receptor molecules useful for practicing the present invention may be prepared by cloning and expression in a prokaryotic or eukaryotic expression system. The recombinant receptor gene may be expressed and purified utilizing any number of methods. The gene encoding the factor
5 may be subcloned into a bacterial expression vector, such as for example, but not by way of limitation, pCP110.

The recombinant factors may be purified by any technique which allows for the subsequent formation of a stable, biologically active protein. For
10 example, and not by way of limitation, the factors may be recovered from cells either as soluble proteins or as inclusion bodies, from which they may be extracted quantitatively by 8M guanidinium hydrochloride and dialysis. In order to further purify the factors, conventional ion exchange chromatography, hydrophobic interaction chromatography, reverse phase
15 chromatography or gel filtration may be used.

The sR α : β heterodimeric receptors may be engineered using known fusion regions, as described in published PCT application WO 93/10151 published May 27, 1993 entitled "Receptor for Oncostatin M and Leukemia Inhibitory
20 Factor" which describes production of β receptor heterodimers, or they may be prepared by crosslinking of extracellular domains by chemical means. The domains utilized may consist of the entire extracellular domain of the α and β components, or they may consist of mutants or fragments thereof that maintain the ability to form a complex with its
25 ligand and other components in the sR α : β 1 complex. For example, as described below in Example 4, IL-6 antagonists have been prepared using gp130 that is lacking its three fibronectin-like domains.

In one embodiment of the invention, the extracellular domains are
30 engineered using leucine zippers. The leucine zipper domains of the human transcription factors c-jun and c-fos have been shown to form stable heterodimers [Busch and Sassone-Corsi, Trends Genetics 6: 36-40

(1990); Gentz, et al., Science 243: 1695-1699 (1989)] with a 1:1 stoichiometry. Although jun-jun homodimers have also been shown to form, they are about 1000-fold less stable than jun-fos heterodimers. Fos-fos homodimers have not been detected.

5

The leucine zipper domain of either c-jun or c-fos are fused in frame at the C-terminus of the soluble or extracellular domains of the above mentioned receptor components by genetically engineering chimeric genes. The fusions may be direct or they may employ a flexible linker domain, such as the hinge region of human IgG, or polypeptide linkers consisting of small amino acids such as glycine, serine, threonine or alanine, at various lengths and combinations. Additionally, the chimeric proteins may be tagged by His-His-His-His-His-His (His6), [SEQ. ID NO. 1] to allow rapid purification by metal-chelate chromatography, and/or by epitopes to which antibodies are available, to allow for detection on western blots, immunoprecipitation, or activity depletion/blocking in bioassays.

In another embodiment, as described below in Example 3, the sR α : β 1 heterodimer is prepared using a similar method, but using the Fc-domain of human IgG1 [Aruffo, et al., Cell 67:35-44 (1991)]. In contrast to the latter, formation of heterodimers must be biochemically achieved, as chimeric molecules carrying the Fc-domain will be expressed as disulfide-linked homodimers. Thus, homodimers may be reduced under conditions that favor the disruption of inter-chain disulfides but do not effect intra-chain disulfides. Then monomers with different extracellular portions are mixed in equimolar amounts and oxidized to form a mixture of homo- and heterodimers. The components of this mixture are separated by chromatographic techniques. Alternatively, the formation of this type of heterodimers may be biased by genetically engineering and expressing molecules that consist of the soluble or extracellular portion of the receptor components followed by the Fc-domain of hIgG, followed by

- either the c-jun or the c-fos leucine zippers described above [Kostelny, et al., J. Immunol. 148: 1547-1553 (1992)]. Since these leucine zippers form predominately heterodimers, they may be used to drive formation of the heterodimers where desired. As for the chimeric proteins described using
- 5 leucine zippers, these may also be tagged with metal chelates or an epitope. This tagged domain can be used for rapid purification by metal-chelate chromatography, and/or by antibodies, to allow for detection on western blots, immunoprecipitation, or activity depletion/blocking in bioassays.
- 10 In additional embodiments, heterodimers may be prepared using other immunoglobulin derived domains that drive the formation of dimers. Such domains include, for example, the heavy chains of IgG (C γ 1 and C γ 4), as well as the constant regions of kappa (κ) and lambda (λ) light chains of human immunoglobulins. The heterodimerization of C γ with the light
- 15 chain occurs between the CH1 domain of C γ and the constant region of the light chain (CL), and is stabilized by covalent linking of the two domains via a single disulfide bridge. Accordingly, as described in Example 4, constructs may be prepared using these immunoglobulin domains. Alternatively, the immunoglobulin domains include domains that may
- 20 be derived from T cell receptor components which drive dimerization. In another embodiment of the invention, the sR α : β 1 heterodimers are prepared by expression as chimeric molecules utilizing flexible linker loops. A DNA construct encoding the chimeric protein is designed such that it expresses two soluble or extracellular domains fused together in
- 25 tandem ("head to head") by a flexible loop. This loop may be entirely artificial (e.g. polyglycine repeats interrupted by serine or threonine at a certain interval) or "borrowed" from naturally occurring proteins (e.g. the hinge region of hIgG). Molecules may be engineered in which the order of the soluble or extracellular domains fused is switched (e.g.
- 30 sIL6R α /loop/sgp130 or sgp130/loop/sIL-6R α) and/or in which the length

and composition of the loop is varied, to allow for selection of molecules with desired characteristics.

Alternatively, the heterodimers made according to the present invention
5 may be purified from cell lines cotransfected with the appropriate α and β components. Heterodimers may be separated from homodimers using methods available to those skilled in the art. For example, limited quantities of heterodimers may be recovered by passive elution from preparative, nondenaturing polyacrylamide gels. Alternatively,
10 heterodimers may be purified using high pressure cation exchange chromatography. Excellent purification has been obtained using a Mono S cation exchange column.

In addition to sR α : β 1 heterodimers that act as antagonists by binding free
15 CNTF or IL-6, the present invention also contemplates the use of engineered, mutated versions of IL-6 with novel properties that allow it to bind to IL-6R α and a single gp130 molecule, but fail to engage the second gp130 to complete β component homodimerization, and thus act as an effective IL-6 antagonist on any IL-6 responsive cell. Our model for the
20 structure of the IL-6 and CNTF receptor complexes indicates that these cytokines have distinct sites for binding the α , β 1, and β 2 receptor components [Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. Mutations of critical amino acid residues comprising each of these sites gives rise to novel molecules which have the desired antagonistic properties. Ablation
25 of the β 1 site would give a molecule which could still bind to the α receptor component but not the β 1 component, and thereby comprise an antagonist with nanomolar affinity. Mutations of critical amino acid residues comprising the β 2 site of IL-6 (IL-6 β 2-) would give a molecule that would bind to IL-6R α and the first gp130 monomer, but fail to engage the
30 second gp130 and thus be functionally inactive. Similarly, mutations of

the CNTF $\beta 2$ site would give a molecule (CNTF $\beta 2^-$) that would bind CNTFR α and gp130, but fail to engage LIFR β , thereby antagonizing CNTF action by forming the non-functional $\beta 1$ intermediate. Based on the binding results described above where CNTF forms the $\beta 1$ intermediate
5 with high affinity, both CNTF $\beta 2^-$ and IL-6 $\beta 2^-$ would constitute antagonists with affinity in the range of 10 pM.

A variety of means are used to generate and identify mutations of IL-6 or CNTF that have the desired properties. Random mutagenesis by standard
10 methods of the DNA encoding IL-6 or CNTF may be used, followed by analysis of the collection of products to identify mutated cytokines having the desired novel properties as outlined below. Mutagenesis by genetic engineering has been used extensively in order to elucidate the structural organization of functional domains of recombinant proteins. Several
15 different approaches have been described in the literature for carrying out deletion or substitution mutagenesis. The most successful appear to be alanine scanning mutagenesis [Cunningham and Wells (1989), Science 244: 1081-1085] and homolog-scanning mutagenesis [Cunningham, et al., (1989), Science 243:1330-1336].

20 Targeted mutagenesis of the IL-6 or CNTF nucleic acid sequences using such methods can be used to generate CNTF $\beta 2^-$ or IL-6 $\beta 2^-$ candidates. The choice of regions appropriate for targeted mutagenesis is done systematically, or determined from studies whereby panels of monoclonal
25 antibodies against each factor are used to map regions of the cytokine that might be exposed after binding of the cytokine to the α receptor component alone, or to the $\alpha\beta 1$ heterodimeric soluble receptors described above. Similarly, chemical modification or limited proteolysis of the cytokine alone or in a complex bound to the α receptor component or the
30 $\alpha\beta 1$ heterodimeric soluble receptors described above, followed by analysis

of the protected and exposed regions could reveal potential $\beta 2$ binding sites.

Assays for identifying CNTF or IL-6 mutants with the desired properties
5 involve the ability to block with high affinity the action of IL-6 or CNTF
on appropriately responsive cell lines [Davis, et al., Science 259: 1736-1739
(1993); Murakami, et al., Proc. Natl. Acad. Sci. USA 88: 11349-11353 (1991)].
Such assays include cell proliferation, survival, or DNA synthesis driven
by CNTF or IL-6, or the construction of cell lines where binding of factor
10 induces production of reporters such as CAT or β -galactosidase [Savino, et
al., Proc. Natl. Acad. Sci. USA 90: 4067-4071 (1993)].

Alternatively, the properties of various mutants may be assessed with a
receptor-based assay. One such assay consists of screening mutants for
15 their ability to bind the sR α : $\beta 1$ receptor heterodimers described above
using epitope-tagged [Davis et al., Science 253: 59-63 (1991)] sR α : $\beta 1$ reagents.
Furthermore, one can probe for the presence or absence of the $\beta 2$ site by
assessing whether an epitope-tagged soluble $\beta 2$ reagent will bind to the
cytokine in the presence of the $\beta 1$ heterodimer. For example, CNTF only
20 binds to LIFR β (the $\beta 2$ component) in the presence of both CNTFR α and
gp130 [Davis, et al. Science 260: 1805-1808 (1993); Stahl, et al. J. Biol. Chem.
268: 7628-7631 (1993)]. Thus a soluble LIFR β reagent would only bind to
CNTF in the presence of the soluble sR α : $\beta 1$ dimer sCNTFR α : $\beta 1$. For IL-6,
the sR α : $\beta 1$ reagent would be IL-6R α : $\beta 1$, and the probe for the $\beta 2$ site would
25 be epitope-tagged sgp130. Thus $\beta 2^-$ mutants of CNTF would be identified
as those that bound the sR α : $\beta 1$ reagent, demonstrating that the α and $\beta 1$
site of the cytokine were intact, yet failed to bind the $\beta 2$ reagent.

In addition, the present invention provides for methods of detecting or measuring the activity of potential β 2- mutants by measuring the phosphorylation of a β -receptor component or a signal transduction component selected from the group consisting of Jak1, Jak2 and Tyk2 or
5 any other signal transduction component, such as the CLIPs, that are determined to be phosphorylated in response to a member of the CNTF family of cytokines.

A cell that expresses the signal transduction component(s) described
10 herein may either do so naturally or be genetically engineered to do so. For example, Jak1 and Tyk-2-encoding nucleic acid sequences obtained as described in Velazquez, et al., Cell, Vol. 70:313-322 (1992), may be introduced into a cell by transduction, transfection, microinjection, electroporation, via a transgenic animal, etc., using any known method
15 known in the art.

According to the invention, cells are exposed to a potential antagonist and the tyrosine phosphorylation of either the β -component(s) or the signal transduction component(s) are compared to the tyrosine phosphorylation
20 of the same component(s) in the absence of the potential antagonist. In another embodiment of the invention, the tyrosine phosphorylation that results from contacting the above cells with the potential antagonist is compared to the tyrosine phosphorylation of the same cells exposed to the parental CNTF family member. In such assays, the cell must either express
25 the extracellular receptor (α -component) or the cells may be exposed to the test agent in the presence of the soluble receptor component. Thus, for example, in an assay system designed to identify agonists or antagonists of CNTF, the cell may express the α - component CNTFR α , the β - components gp130 and LIFR β and a signal transducing component such as
30 Jak1. The cell is exposed to test agents, and the tyrosine phosphorylation of either the β - components or the signal transducing component is

compared to the phosphorylation pattern produced in the presence of CNTF. Alternatively, the tyrosine phosphorylation which results from exposure to a test agent is compared to the phosphorylation which occurs in the absence of the test agent. Alternatively, an assay system, for
5 example, for IL-6 may involve exposing a cell that expresses the β -component gp130 and a signal transducing protein such as Jak1, Jak2 or Tyk2 to a test agent in conjunction with the soluble IL-6 receptor.

In another embodiment of the invention the above approaches are used to
10 develop a method for screening for small molecule antagonists that act at various steps in the process of ligand binding, receptor complex formation, and subsequent signal transduction. Molecules that potentially interfere with ligand-receptor interactions are screened by assessing interference of complex formation between the soluble receptors and ligand as described
15 above. Alternatively, cell-based assays in which IL-6 or CNTF induce response of a reporter gene are screened against libraries of small molecules or natural products to identify potential antagonists. Those molecules showing antagonist activity are rescreened on cell-based assays responding to other factors (such as GM-CSF or factors like Neurotrophin-
20 3 that activate receptor tyrosine kinases) to evaluate their specificity against the CNTF/IL-6/OSM/LIF family of factors. Such cell-based screens are used to identify antagonists that inhibit any of numerous targets in the signal transduction process.

25 In one such assay system, the specific target for antagonists is the interaction of the Jak/Tyk family of kinases [Firmbach-Kraft, Oncogene 5: 1329-1336 (1990); Wilks, et al., Mol. Cell. Biol. 11:2057-2065 (1991)] with the receptor β subunits. As described above, LIFR β and gp130 preassociate with members of the Jak/Tyk family of cytoplasmic protein tyrosine
30 kinases, which become activated in response to ligand-induced β component dimerization Stahl, et al. Science 263:92-95 (1993). Thus small molecules that could enter the cell cytoplasm and disrupt the interaction

between the β component and the Jak/Tyk kinase could potentially block all subsequent intracellular signaling. Such activity could be screened with an *in vitro* scheme that assessed the ability of small molecules to block the interaction between the relevant binding domains of purified β component and Jak/Tyk kinase. Alternatively, one could easily screen for molecules that could inhibit a yeast-based assay of β component binding to Jak/Tyk kinases using the two-hybrid interaction system [Chien, et al., Proc. Natl. Acad. Sci. 88: 9578-9582 (1991)]. In such a system, the interaction between two proteins (β component and Jak/Tyk kinase or relevant domains thereof in this example) induces production of a convenient marker such as β -galactosidase. Collections of small molecules are tested for their ability to disrupt the desired interaction without inhibiting the interaction between two control proteins. The advantage of this screen would be the requirement that the test compounds enter the cell before inhibiting the interaction between the β component and the Jak/Tyk kinase.

The CNTF family antagonists described herein either bind to, or compete with the cytokines CNTF and IL-6. Accordingly, they are useful for treating diseases or disorders mediated by CNTF or IL-6. For example, therapeutic uses of IL-6 antagonists would include the following:

1) In osteoporosis, which can be exacerbated by lowering of estrogen levels in post-menopausal women or through ovariectomy, IL-6 appears to be a critical mediator of osteoclastogenesis, leading to bone resorption [Horowitz, Science 260: 626-627 (1993); Jilka, et al., Science 257: 88-91 (1992)]. Importantly, IL-6 only appears to play a major role in the estrogen-depleted state, and apparently is minimally involved in normal bone maintenance. Consistent with this, experimental evidence indicates that function-blocking antibodies to IL-6 can reduce the number of osteoclasts [Jilka, et al. Science 257: 88-91 (1992)]. While estrogen replacement therapy is also used, there appear to be side effects that may include increased risk of

endometrial and breast cancer. Thus, IL-6 antagonists as described herein would be more specific to reduce osteoclastogenesis to normal levels.

2) IL-6 appears to be directly involved in multiple myeloma by acting in either an autocrine or paracrine fashion to promote tumor formation [van Oers, et al., *Ann Hematol.* 66: 219-223 (1993)].
Furthermore, the elevated IL-6 levels create undesirable secondary effects such as bone resorption, hypercalcemia, and cachexia; in limited studies function-blocking antibodies to IL-6 or IL-6Ra have some efficacy [Klein, et al., *Blood* 78: 1198-1204 (1991); Suzuki, et al., *Eur. J. Immunol.* 22:1989-1993 (1992)]. Therefore, IL-6 antagonists as described herein would be beneficial for both the secondary effects as well as for inhibiting tumor growth.

3) IL-6 may be a mediator of tumor necrosis factor (TNF) that leads to cachexia associated with AIDS and cancer [Strassmann, et al., *J. Clin. Invest.* 89: 1681-1684 (1992)], perhaps by reducing lipoprotein lipase activity in adipose tissue [Greenberg, et al., *Cancer Research* 52: 4113-4116 (1992)]. Accordingly, antagonists described herein would be useful in alleviating or reducing cachexia in such patients.

Effective doses useful for treating these or other CNTF family related diseases or disorders may be determined using methods known to one skilled in the art [see, for example, Fingl, et al., *The Pharmacological Basis of Therapeutics*, Goodman and Gilman, eds. Macmillan Publishing Co., New York, pp. 1-46 ((1975))]. Pharmaceutical compositions for use according to the invention include the antagonists described above in a pharmacologically acceptable liquid, solid or semi-solid carrier, linked to a carrier or targeting molecule (e.g., antibody, hormone, growth factor, etc.) and/or incorporated into liposomes, microcapsules, and controlled release preparation (including antagonist expressing cells) prior to administration *in vivo*. For example, the pharmaceutical composition may comprise one or more of the antagonists in an aqueous solution, such as sterile water, saline, phosphate buffer or dextrose solution. Alternatively, the active agents may be comprised in a solid (e.g. wax) or semi-solid (e.g. gelatinous) formulation that may be implanted into a patient in need of such

treatment. The administration route may be any mode of administration known in the art, including but not limited to intravenously, intrathecally, subcutaneously, by injection into involved tissue, intraarterially, intranasally, orally, or via an implanted device.

5

Administration may result in the distribution of the active agent of the invention throughout the body or in a localized area. For example, in some conditions which involve distant regions of the nervous system, intravenous or intrathecal administration of agent may be desirable. In some situations, an implant containing active agent may be placed in or near the lesioned area. Suitable implants include, but are not limited to, gelfoam, wax, or microparticle-based implants.

10

EXAMPLES

15

EXAMPLE 1: CNTF COMPETES WITH IL-6 FOR BINDING TO GP130

MATERIALS AND METHODS

Materials. A clone of PC12 cells that respond to IL-6 (PC12D) was obtained from DNAX. Rat CNTF was prepared as described [Masiakowski, et al., J. Neurochem. 57:1003-10012 (1991)]. IL-6 and sIL-6R α were purchased from R & D Systems. Antisera was raised in rabbits against a peptide derived from a region near the C-terminus of gp130 (sequence: CGTEGQVERFETVGME) [SEQ. ID. NO. 2] by the method described (Stahl, et al. J. Biol. Chem. 268:7628-7631 (1993). Anti-phosphotyrosine monoclonal 4G10 was purchased from UBI, and reagents for ECL from Amersham.

20

Signal Transduction Assays. Plates (10 cm) of PC12D were starved in serum-free medium (RPMI 1640 + glutamine) for 1 hour, then incubated with IL-6 (50 ng/mL) + sIL-6R (1 mg/mL) in the presence or absence of

added rat CNTF at the indicated concentrations for 5 minutes at 37°C. Samples were then subjected to anti-gp130 immunoprecipitation, SDS PAGE, and anti-phosphotyrosine immunoblotting as described (Stahl, et al. J. Biol. Chem. 268:7628-7631 (1993)).

5

RESULTS

The ability of CNTF to block IL-6 responses was measured using a PC12 cell line (called PC12D) that expresses IL-6R α , gp130, and CNTFR α , but not LIFR β . As one would predict, these cells respond to IL-6, but not to CNTF (Fig. 2) since LIFR β is a required component for CNTF signal transduction [Davis, et al., Science 260: 59-63 (1993)]. In accordance with results on other cell lines [Ip, et al., Cell 69: 1121-1132 (1992)], PC12D cells give tyrosine phosphorylation of gp130 (as well as a variety of other proteins called CLIPs) in response to 2 nM IL-6 (Fig. 2). Addition of recombinant soluble IL-6R α (sIL-6R α) enhances the level of gp130 tyrosine phosphorylation, as has been reported in some other systems [(Taga, et al., Cell 58: 573-581 (1989)]. However, addition of 2 nM CNTF simultaneously with IL-6 severely diminishes the tyrosine phosphorylation of gp130. Although a slight gp130 phosphorylation response remains in the presence of CNTF, IL-6, and sIL-6R α , it is eliminated if the CNTF concentration is increased fourfold to 8 nM. Thus, in IL-6 responsive cells that contain CNTFR α but no LIFR β , CNTF is a rather potent antagonist of IL-6 action.

25 EXAMPLE 2. BINDING OF CNTF TO THE CNTFR α : β

MATERIALS AND METHODS

Scatchard Analysis of CNTF Binding. ¹²⁵I-CNTF was prepared and purified as described [Stahl et al. JBC 268: 7628-7631 (1993)]. Saturation binding studies were carried out in PC12 cells, using concentrations of ¹²⁵I-

30

CNTF ranging from 20pM to 10nM. Binding was performed directly on a monolayer of cells. Medium was removed from wells and cells were washed once with assay buffer consisting of phosphate buffered saline (PBS; pH 7.4), 0.1mM bacitracin, 1mM PMSF, 1mg/ml leupeptin, and
5 1mg/ml BSA. Cells were incubated in ^{125}I -CNTF for 2 hours at room temperature, followed by 2 quick washes with assay buffer. Cells were lysed with PBS containing 1% SDS and counted in a Packard Gamma Counter at 90-95% efficiency. Non-specific binding was defined by the presence of 100-fold excess of unlabelled CNTF. Specific binding ranged
10 from 70% to 95%.

RESULTS

The equilibrium constant for binding of CNTF to CNTFR α : β 1 was
15 estimated from Scatchard analysis of iodinated CNTF binding on PC12D cells (Figure 3). The data is consistent with a 2 site fit having dissociation constants of 9 pM and 3.4 nM. The low affinity site corresponds to interaction of CNTF with CNTFR α , which has a K_d near 3 nM [(Panayotatos, et al., J. Biol. Chem. 268: 19000-19003 (1993))]. We interpret
20 the high affinity complex as the intermediate containing CNTF, CNTFR α , and gp130. A Ewing sarcoma cell line (EW-1) which does contain CNTFR α , gp130, and LIFR β , and therefore gives robust tyrosine phosphorylation in response to CNTF, displays a very similar two site fit with dissociation constants of 1 nM and 10. Thus it is apparent that CNTF
25 binds with equally high affinity to a complex containing only CNTFR α and gp130, as it does to a complex which additionally contains LIFR β , thus demonstrating the feasibility of creating the sR α : β antagonists described herein.

EXAMPLE 3. METHODS OF PRODUCING CYTOKINE LIGAND TRAPS

Virus Stock Production

- 5 SF21 insect cells obtained from *Spodoptera frugiperda* were grown at 27°C in Gibco SF900 II medium to a density of 1×10^6 cells/mL. The individual virus stock for either GP130-Fc-His6 (Figure 4) or IL6Ra-Fc (Figure 5) was added to the bioreactor to a low multiplicity 0.01-0.1 PFU/cell to begin the infection. The infection process was allowed to continue for 5-7 days
- 10 allowing maximum virus replication without incurring substantial cell lysis. The cell suspension was aseptically aliquoted into sterile centrifuge bottles and the cells removed by centrifugation. The cell-free supernatant was collected in sterile bottles and stored at 4°C until further use.
- 15 The virus titer was determined by plaque assay as described by O'Reilly, Miller and Luckow. The method is carried out in 60mm tissue-culture dishes which are seeded with 2×10^6 cells. Serial dilutions of the virus stock are added to the attached cells and the mixture incubated with rocking to allow the virus to adsorb to individual cells. An agar overlay is
- 20 added and plates incubated for 5 - 7 days at 27°C. Staining of viable cells with neutral red revealed circular plaques resulting which were counted to give the virus titer.

Coinfection of Cells for Protein Production

- 25 Uninfected SF21 Cells were grown in a 60L ABEC bioreactor containing 40L of SF900 II medium. Temperature was controlled at 27°C and the dissolved oxygen level was maintained at 50% of saturation by controlling the flowrate of oxygen in the inlet gas stream. When a density of 2×10^6
- 30 cells/mL was reached, the cells were concentrated within the bioreactor to a volume of 20L using a low shear steam sterilizable pump with a tangential flow filtration device with Millipore ProstaK 0.65 micron

membranes. After concentration fresh sterile growth medium is slowly added to the bioreactor while the filtration system continues to remove the spent growth medium by diafiltration. After two volume exchanges (40L) have been carried out an additional 20L of fresh medium was added
5 to the bioreactor to resuspend the cells to the original volume of 40L. The cell density was determined once again by counting viable cells using a hemacytometer.

The required amount of each virus stock was calculated based on the cell
10 density, virus titer and the desired multiplicity of infection (MOI). Virus stock ratios of 5:1, 5:2, 10:2 and 10:4, IL6R α -Fc to GP130-Fc-His₆ all resulted in production of significant amounts of heterodimer. The ideal virus stock ratio is highly dependent on the ease of purification of the heterodimer from each of the two homodimers. The IL6R α -Fc
15 homodimer is relatively easy to remove downstream by immobilized metal affinity chromatography. Virus infection ratios have been chosen to minimize the formation of the GP130-Fc-His₆ homodimer which is more difficult to clear downstream. The relative amount of GP130-Fc-His₆ virus stock chosen for infection has increased with successive batches as the
20 purification method for clearing the resultant homodimer has improved.

The virus stocks were aseptically mixed in a single vessel then transferred to the bioreactor. This results in synchronous infection of the SF21 cells. The infection is allowed to proceed for three to four days, allowing
25 sufficient time for maximal production of the heterodimer protein.

Recovery and Protein A Chromatographic Purification

At the conclusion of the infection phase of the bioreactor process the cells
30 were concentrated in the bioreactor using a 10 ft² Millipore Prostack filter (0.65 micron) pore size. The cell-free permeate passing through the filter was collected in a clean process vessel. At the conclusion of the filtration

operation the pH of permeate stream, containing the protein product, was adjusted to 8.0 with 10N NaOH. The resultant precipitate was removed by forcing the extract through a 0.8 micron depth filter (Sartorius), followed by a 0.2 micron filter. Sufficient 0.5M EDTA stock was added to give a final
5 concentration of 5mM. The filtered protein solution was loaded onto a 10 cm diameter column containing 100-200 mL of Pharmacia Protein A Sepharose 4 Fast Flow, equilibrated with PBS. Protein A has a very high affinity for the Fc-Fc domain of each of the 3 recombinant protein products, allowing them to bind while other proteins in the cell-free
10 extract flow through the column. After loading the column was washed to baseline with PBS containing an additional 350mM NaCl. The IgG-Fc tagged proteins were eluted at low pH, either with 0.5M acetic acid or with a decreasing pH gradient of 0.1M citric acid and 0.2M disodium phosphate buffers. Tris base or disodium phosphate was added to the eluted protein
15 to avoid prolonged exposure to low pH conditions.

The pooled protein was diafiltered into PBS or HEPES buffer and derivitized with 1 mM iodoacetamide to protect the exposed sulfhydryl group on the free cysteine near the hinge region of each Fc domain. This
20 prevents disulfide mediated aggregation of proteins. A 6 ft² Millipore spiral wound ultrafiltration membrane with nominal 30 kiloDalton cutoff was used to perform the buffer exchange. The total protein was determined by UV absorbance at 280 nm using the diafiltration buffer as a blank. The relative amounts of heterodimer and two homodimer
25 proteins were determined by SDS PAGE gel electrophoresis using a 6% Tris-Glycine gel (Novex). Gels were Coomassie-stained then transferred into destain solution overnight. A Shimadzu scanning densitometer was used to determine the relative intensity of the individual protein bands on the SDS PAGE gel. The peak area ratios are used to compute the fraction of
30 heterodimer and each of the homodimers in the column pool fractions.

Immobilized Metal Affinity Chromatographic Purification

The six histidine residues on the C-terminus of the GP130-Fc-His₆ fusion protein provides an excellent molecular handle for separation of the
5 heterodimeric IL6 antagonist from the two homodimers. The imidazole group on each of the C-terminal histidines of the GP130-Fc-His₆ moiety has a strong binding constant with several divalent metals, including copper, nickel, zinc, cobalt, iron and calcium. Since the IL6R α -Fc homodimer has no C-terminal histidine residues, it clearly has the lowest
10 affinity. The IL6R α -Fc-GP130-Fc-His₆ heterodimer has a single stand set six histidines giving it greater affinity for the metal, while the GP130-Fc-His₆ homodimer has two sets of six histidines each giving it the highest affinity of the three IgG tagged proteins to the metal affinity column. Selective elution of the three proteins with increasing amounts of
15 imidazole in the elution buffer therefore elutes the proteins in the following order:

1. IL6R α -Fc homodimer
2. IL6R α -Fc-GP130-Fc-His heterodimer
- 20 3. GP130-Fc-His homodimer

A 26 mm diameter column containing 100 mL of Pharmacia Chelating Sepharose Fast Flow was saturated with a solution of nickel sulfate until a
25 significant green color is observed in the column eluate. The column is then washed with several column volumes of deionized water, then equilibrated with 50 mM HEPES, 40mM imidazole, pH 8.0. The binding of imidazole to the immobilized nickel results in a green to blue color change. Imidazole was added to the protein load to a final concentration of 40mM. Addition of imidazole to the protein load reduces the binding of
30 IL6R α -Fc homodimer, increasing the surface area available for the remaining two species. After loading, the column was washed with

several column volumes of 50 mM HEPES, 80mM imidazole, pH 8.0 until a steady baseline was reestablished. The heterodimer was selectively eluted with 50 mM HEPES, 150mM imidazole, pH 8.0 over several column volumes. The protein fractions were pooled and diafiltered into PBS as
5 described in the section above.

EXAMPLE 4. ALTERNATIVE METHODS OF CONSTRUCTING LIGAND TRAPS

10 As described above, receptor activation by CNTF, and analogously by IL-6 and IL-11, follows an ordered sequence of binding events (Figure 6). The cytokine initially binds to its cognate $R\alpha$ with low affinity ($K_d = 3$ to 10 nM); this is a required step - cells which do not express the cognate $R\alpha$ do not respond to the cognate cytokine. The cytokine• $R\alpha$ complex associates
15 with the first signal transducing component, gp130, to form a high affinity complex (K_d in the order of 10 pM for the CNTF•CNTF $R\alpha$ •gp130 complex). This complex does not transduce signal, as it is the dimerization of the signal transducing components that brings about signaling (Stahl and Yancopoulos, J. Neurobiology 25: 1454-1466 (1994); Stahl et al., Science
20 267:1349-1353 (1995); Davis et al., Science 260:1805-1808 (1993); Stahl et al., Science 263:92-95 (1994); Murakami, et al. Science 260:1808-1810 (1993). At least in the case of IL-6, the cytokine• $R\alpha$ •signal transducer heterotrimeric complex subsequently associates with another like complex, to form a hexameric complex (Figure 6) (Ward et al., J. Biol. Chem. 269:23286-23289
25 (1994). The resulting dimerization of the signal transducers - gp130 in the case of IL-6 (Murakami et al., Science 260:1808-1810 (1993) and IL-11, gp130 and LIFR in the case of CNTF (Davis et al., Science 260:1805-1808 (1993) - brings about signal transduction.

30 The initial heterodimeric molecules made comprised a soluble $R\alpha$ -component linked to the extracellular domain of gp130. These molecules

were shown to mimic the high affinity cytokine•R α •gp130 complex and behave as a high affinity antagonist of their cognate cytokine (Figure 7). To make these molecules, the extracellular domain of gp130 was paired with the extracellular domain of the α -receptor components for IL-6 and CNTF, IL-6R α and CNTFR α respectively. To link the R α with the extracellular domain of gp130, the soluble R α -components and gp130 were fused to the Fc portion of human IgG1 to produce R α -Fc and gp130-Fc respectively. The Fc domain was chosen primarily but not solely because it naturally forms disulfide-linked dimers. Heterodimeric molecules comprising R α -Fc•gp130-Fc were expressed, purified and shown to behave as highly potent antagonists of their cognate ligand. Furthermore, these molecules were found to be highly specific for their cognate cytokine since it is the choice of the α -receptor component which specifies which cytokine is bound and trapped (there is no measurable binding of the cytokine to gp130 in the absence of the appropriate R α).

Here we describe an extension of this technology which allows the engineering of different heteromeric soluble receptor ligand traps which by virtue of their design may have additional beneficial characteristics such as stability, Fc-receptor-mediated clearance, or reduced effector functions (such as complement fixation). Furthermore, the technology described should prove suitable for the engineering of any heteromeric protein in mammalian or other suitable protein expression systems, including but not limited to heteromeric molecules which employ receptors, ligands, and catalytic components such as enzymes or catalytic antibodies.

MATERIALS AND METHODS

Genetic engineering of heteromeric immunoglobulin heavy/light chain soluble receptor-based ligand traps for IL-6.

The IL-6 traps described here were engineered using human gp130, human IL-6 α -receptor (IL-6R α), the constant region of the heavy chains (C γ) of human IgG1 (C γ 1) (Lewis et al., Journal of Immunology 151:2829-2838 (1993) or IgG4 (C γ 4) with or without a join-region (J), and the constant regions of kappa (κ) and lambda (λ) (Cheung, et al., Journal of Virology 66:6714-6720 (1992) light chains of human immunoglobulin (Ig), also with or without a different j-peptide (j)). This design takes advantage of the natural ability of the C γ domain to heterodimerize with κ or λ light chains. The heterodimerization of C γ with the light chain occurs between the CH1 domain of C γ and the constant region of the light chain (C L), and is stabilized by covalent linking of the two domains via a single disulfide bridge. We reasoned that, like the Fc domain of human IgG1, the combination of C γ with C L could be used to produce disulfide linked heteromeric proteins comprised of the extracellular domain of gp130 on one chain and the extracellular domain of IL-6R α on the other chain. Like their Fc-based counterparts, such proteins were postulated to be high affinity ligand traps for IL-6 and as a result to inhibit the interaction of IL-6 with the native receptor on IL-6-responsive cells, thus functioning as IL-6 antagonists. Furthermore, constructs employing the full length C γ region would, much like antibodies, form homodimers of the C γ chain, giving rise to antibody-like molecules comprising of two "light chains" and two "heavy chains" (Figure 8). The potential advantage of this design is that it may more closely mimic the IL-6•IL-6R α •gp130 complex and may display a higher affinity for the ligand than comparable single heterodimers. An additional design is incorporated by using truncated versions of C γ , comprised only of the CH1 domain. These will form heterodimeric molecules with receptor- κ fusion proteins, and will thus resemble the Fab fragment of antibodies.

All the soluble receptor-Ig chimeric genes may be engineered in plasmid vectors including, but not limited to, vectors suitable for mammalian expression (COS monkey kidney cells, Chinese Hamster Ovary cells [CHO], and ras-transformed fibroblasts [MG-ras]) and include a Kozak sequence (CGC CGC CAC CAT GGT G) at the beginning of each chimeric gene for efficient translation. Engineering was performed using standard genetic engineering methodology. Each construct was verified by DNA sequencing, mammalian expression followed by western blotting with suitable antibodies, biophysical assays that determine ligand binding and dissociation, and by growth inhibition assays (XG-1, as described later). Since the domains utilized to engineer these chimeric proteins are flanked by appropriate restriction sites, it is possible to use these domains to engineer other chimeric proteins, including chimeras employing the extracellular domains of the receptors for factors such as IL-1, IL-2, IL-3, IL-4, IL-5, GM-CSF, LIF, IL-11, IL-15, IFN γ , TGF β , and others. The amino acid coordinates for each component utilized in making the IL-6 traps are listed below (Note: numbering starts with the initiating methionine as #1; long sequences are listed using the single letter code for the twenty amino acids):

20

(a) Constructs employing human gp130:

- (i) **gp130-C γ 1** was engineered by fusing in frame the extracellular domain of gp130 (amino acids 1 to 619) to a Ser-Gly bridge, followed by the 330 amino acids which comprise C γ 1 and a termination codon (Figure 9).
- 25 (ii) **gp130-J-C γ 1** was engineered in the same manner as gp130-C γ 1 except that a J-peptide (amino acid sequence: GQGTLVTVSS) was inserted between the Ser-Gly bridge and the sequence of C γ 1 (see Figure 9).
- (iii) **gp130 Δ 3fibro-C γ 1** was engineered by fusing in frame the extracellular domain of gp130 without its three fibronectin-like domains (Figure 10).
- 30 The remaining part of this chimeric protein is identical to gp130-C γ 1.

- (iv) **gp130-J-CH1** was engineered in a manner identical for that described for gp130-C γ 1, except that in place of the C γ 1 region only the CH1 part of C γ 1 has been used (Figure 11). The C-terminal domain of this construct includes the part of the hinge that contains the cysteine residue
5 responsible for heterodimerization of the heavy chain of IgG with a light chain. The part of the hinge that contains the two cysteines involved in C γ 1 homodimerization has been deleted along with the CH2 and CH3 domains.
- (v) **gp130-C γ 4** was engineered in a manner identical to that described for
10 gp130-C γ 1, except that C γ 4 was used in place of C γ 1 (Figure 12). In addition, an *RsrII* DNA restriction site was engineered at the hinge region of the C γ 4 domain by introducing two silent base mutations. The *RsrII* site allows for other desired genetic engineering manipulations, such as the construction of the CH1 equivalent of gp130-C γ 4.
- 15 (vi) **gp130- κ** was engineered in a manner identical to that described for gp130-C γ 1, except that the constant region of the κ light chain of human Ig was used in place of C γ 1 (Figure 13).
- (vi) **gp130-J- κ** was engineered in a manner identical to that described for gp130-J- κ , except that a j-peptide (amino acid sequence: TFGQGTKVEIK)
20 was inserted between the Ser-Gly bridge and the κ -region.
- (viii) **gp130- λ** was engineered in a manner identical to that described for gp130-C γ 1, except that the constant region of the λ light chain (Cheung, et al., Journal of Virology 66:6714-6720 (1992) of human Ig was used in place of C γ 1 (Figure 14).
25
- (b) Constructs employing human IL-6R α :**
- (i) **IL6R α -C γ 1** was engineered by fusing in frame amino acids 1 to 358 of IL-6R α (Yamasaki et al., Science 241:825-828 (1988), which comprise the

extracellular domain of IL-6R α (Figure 15), to an Ala-Gly bridge, followed by the 330 amino acids which comprise C γ 1 and a termination codon.

(ii) IL6R α - κ was engineered as described for IL6R α -C γ 1, except that the κ -domain (Figure 13) utilized for gp130- κ was used in place of C γ 1.

5 (iii) IL6R α -j- κ was engineered as described for IL6R α - κ except that the j-peptide described for gp130-j- κ was placed between the Ala-Gly bridge and the κ -domain.

(iv) Three additional constructs, IL6R α 313-C γ 1, IL6R α 313- κ , and IL6R α 313-j- κ , were engineered as using a truncated form of IL-6R α comprised of
10 amino acids 1 to 313 (Figure 16). Each of these constructs were made by fusing in frame IL6R α 313 with a Thr-Gly bridge followed by the C γ 1, κ -, and j- κ -domains described above. These constructs were engineered in order to complement the gp130 Δ 3fibro-derived constructs.

15 Expression and purification of ligand traps

To produce covalently linked heterodimers of soluble gp130 and soluble IL-6R α , gp130-Ig chimeric proteins were co-expressed with appropriate IL-6R α -Ig chimeric proteins in complementing pairs. Co-expression was
20 achieved by co-transfecting the corresponding expression vectors into suitable mammalian cell lines, either stably or transiently. The resulting disulfide-linked heterodimers were purified from conditioned media by several different methods, including but not limited to affinity chromatography on immobilized Protein A or Protein G, ligand-based
25 affinity chromatography, ion exchange, and gel filtration.

An example of the type of methods used for purification of a heavy/light receptor fusion protein is as follows: gp130-C γ 1•IL-6R α - κ was expressed in COS cells by co-transfecting two different vectors, encoding gp130-C γ 1 and

IL-6R α - κ respectively. Serum-free conditioned media (400 ml) were collected two days post-transfection and C γ 1-bearing proteins were purified by affinity chromatography over a 1ml Protein A Sepharose (Pharmacia). The material generated in this step was further purified by a second
5 affinity chromatography step over a 1 ml NHS-activated Sepharose (Pharmacia) which was derivatized with recombinant human IL-6, in order to remove gp130-C γ 1 dimer from gp130-C γ 1•IL-6R α - κ complexes (the gp130-C γ 1 dimer does not bind IL-6). Proteins generated by this method were more than 90% pure, as evidenced by SDS-PAGE followed by silver-
10 staining (Figure 17). Similar protocols have been employed successfully towards the purification of other heavy/light receptor heterodimers.

RESULTS

15 Biological activity of immunoglobulin heavy/light chain receptor fusion antagonists

The purified ligand traps were tested for their ability to bind IL-6 in a variety of different assays. For example, the dissociation rate of IL-6 bound
20 to the ligand trap was measured in parallel with the dissociation rate of IL-6 from the anti-IL-6 monoclonal neutralizing antibody B-E8 [Brochier, et al., Int. J. Immunopharmacology 17:41-48 (1995), and references within]. An example of this type of experiment is shown in Figure 18. In this experiment 20 pM ¹²⁵I-IL-6 (1000 μ Ci/mmol; Amersham) was
25 preincubated with 500 pM of either gp130-C γ 1•IL-6R α - κ or mAb B-E8 for 20 hours. At this point a 1000-fold excess (20 nM) of "cold" IL-6 was added. Periodically, aliquots of the reaction were removed, the ligand trap or B-E8 were precipitated with Protein G-Sepharose, and the number of cpm of ¹²⁵I-IL-6 that remained bound was determined. Clearly, the dissociation
30 rate of human ¹²⁵I-IL6 from the ligand trap was very slow - after three days, approximately 75% of the initial counts were still bound to the ligand

trap. In contrast, less than 5% of the counts remained associated with the antibody after three days. This result demonstrates that the dissociation rate of the ligand from these ligand traps is very slow.

- 5 In a different set of experiments the ability of the ligand traps to multimerize in the presence of ligand was tested. An example of this is shown in Figure 19. IL-6-induced association of gp130-Fc•IL-6R α -Fc with gp130-CH1•IL-6R α - κ was determined by testing whether gp130-CH1•IL-6R α - κ , which does not by itself bind Protein A, could be precipitated by
- 10 Protein A-Sepharose in the presence of gp130-Fc•IL-6R α -Fc in an IL-6-dependent manner (Figure 9). Precipitation of gp130-CH1•IL-6R α - κ by Protein A-Sepharose was determined by western blotting with an anti-kappa specific HRP conjugate, which does not detect gp130-Fc•IL-6R α -Fc. gp130-CH1•IL-6R α - κ could be precipitated by Protein A-Sepharose only
- 15 when both gp130-Fc•IL-6R α -Fc and IL-6 were present. This result conclusively indicates that IL-6 can induce ligand trap multimerization, and further indicate that the ligand trap can mimic the hexameric cytokine•R α •signal transducer complex (Figure 1). Ligand-induced multimerization may play a significant role in the clearance of
- 20 cytokine•ligand trap complexes *in vivo*.

- The biological activity of the different ligand traps may be further tested in assays which measure ligand-dependent cell proliferation. Several cell proliferation assays exist for IL-6 and they employ cell lines such as B9,
- 25 CESS, or XG-1. An example of this type of assay using the XG-1 cell line is presented below: XG-1 is a cell line derived from a human multiple myeloma (Zhang, et al., Blood 83:3654-3663 (1994). XG-1 depends on exogenously supplied human IL-6 for survival and proliferation. The EC₅₀ of IL-6 for the XG-1 line is approximately 50 pmoles/ml. The ability of
- 30 several different IL-6 traps to block IL-6-dependent proliferation of XG-1

cells was tested by incubating increasing amounts of purified ligand traps with 50 pg/ml IL-6 in XG-1 cultures. The ligand traps which were tested had been expressed and purified by methods similar to those described above. All of the ligand traps tested were found to inhibit IL-6-dependent proliferation of XG-1 in a dose dependent manner (Figure 20). Of the five different traps tested gp130-C γ 1•IL-6R α - κ was the most active and essentially display the same neutralizing activity towards IL-6 as the antibody B-E8. As little as a 10-fold molar excess of either gp130-C γ 1•IL-6R α - κ or B-E8 completely blocked the activity of IL-6 (a reading of A570-650 = 0.3 AU corresponds to no proliferation of the XG-1 cells). At a 100-fold molar excess all of the ligand traps tested completely blocked the activity of IL-6. This observed inhibition is highly selective as neither a gp130-Fc•CNTFR α -Fc ligand trap which blocks CNTF activity, nor gp130-Fc homodimer exhibit any blocking activity towards IL-6 even when used at a 1000-fold molar excess over IL-6 (data not shown). This data demonstrates that the heteromeric immunoglobulin heavy/light chain receptor-based ligand traps function as selective high affinity antagonists of their cognate ligand.

20 EXAMPLE 5 - CLONING OF FUSION POLYPEPTIDE COMPONENTS

The extracellular domains of the human cytokine receptors were obtained by standard PCR techniques using tissue cDNAs (CLONTECH), cloned into the expression vector, pMT21 (Genetics Institute, Inc.), and the sequences were sequenced by standard techniques using an ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA). For the IL-4R α , nucleotides 241 through 868 (corresponding to the amino acids 24-231) from the Genbank sequence, X52425, were cloned. For the IL-2R γ , nucleotides 15 through 776 (corresponding to amino acids 1-233) from the Genbank sequence, D11086, were cloned. For the IL-6R α , nucleotides 52 through 1044 (corresponding

to the amino acids 1-331) from the Genbank sequence, X52425, were cloned. For gp130, nucleotides 322 through 2112 (corresponding to the amino acids 30-619) from the Genbank sequence, M57230, were cloned. For the IL-1RAcP, nucleotides 1 through 1074 (corresponding to the amino acids 1-358) from the Genbank sequence, AB006357, were cloned. For the IL-1RI, nucleotides 55 through 999 (corresponding to the amino acids 19-333) from the Genbank sequence, X16896, were cloned.

EXAMPLE 6 - PRODUCTION OF FUSION POLYPEPTIDES (CYTOKINE TRAPS)

The nucleotide sequences encoding the cytokine traps were constructed from the individual cloned DNAs (described *supra*) by standard cloning and PCR techniques. In each case, the sequences were constructed in frame such that the sequence encoding the first fusion polypeptide component was fused to the sequence encoding the second fusion polypeptide component followed by an Fc domain (hinge, CH2 and CH3 region of human IgG1) as the multimerizing component. In some cases extra nucleotides were inserted in frame between sequences encoding the first and second fusion polypeptide components to add a linker region between the two components (See Figure 21A - Figure 21D - trap 424; Figure 24A - Figure 24F - trap 412; and Figure 26A - Figure 26E - trap 569).

For the IL-4 traps, 424 (Figure 21A - Figure 21D), 603 (Figure 22A - Figure 22D) and 622 (Figure 23A - Figure 23D), the IL-2R γ component is 5', followed by the IL4R α component and then the Fc component. For the IL-6 traps, 412 (Figure 24A - Figure 24F) and 616 (Figure 25A - Figure 25F), the IL-6R α component is 5' followed by the gp130 component and then the Fc domain. For the IL-1 trap 569 (Figure 26A - Figure 26E), the IL-1RAcP component is 5' followed by the IL-1RI component and then the Fc domain. The final constructs were cloned into the mammalian expression vector pCDNA3.1 (STRATAGENE).

In the 569 sequence (Figure 26A - Figure 26E), nucleotides 1-1074 encode the IL1RAcP component, nucleotides 1075 -1098 encode a linker region, nucleotides 1099-2043 encode the IL1RI component and nucleotides 2044-
5 2730 encode the Fc domain.

In the 412 sequence (Figure 24A - Figure 24F), nucleotides 1-993 encode the IL6R α component, nucleotides 994-1023 encode a linker region, nucleotides 1024-2814 encode the gp130 component and nucleotides 2815-
10 3504 encode the Fc domain.

In the 616 sequence (Figure 25A - Figure 25F), nucleotides 1-993 encode the IL6R α component, nucleotides 994-2784 encode the gp130 component and nucleotides 2785-3474 encode the Fc domain.
15

In the 424 (Figure 21A - Figure 21D) and 622 (Figure 23A - Figure 23D) sequences, nucleotides 1-762 encode the IL2R γ component, nucleotides 763-771 encode a linker region, nucleotides 772-1395 encode the IL4R α component and nucleotides 1396-2082 encode the Fc domain.
20

Finally, in the 603 sequence (Figure 22A - Figure 22D), nucleotides 1-762 encode the IL2R γ component, nucleotides 763-1386 encode the IL4R α component and nucleotides 1387-2073 encode the Fc domain.

25 DNA constructs were either transiently transfected into COS cells or stably transfected into CHO cells by standard techniques well known to one of skill in the art. Supernatants were collected and purified by Protein A affinity chromatography and size exclusion chromatography by standard techniques. (See for example Harlow and Lane, Antibodies - A Laboratory
30 Manual, Cold Spring Harbor Laboratory, 1988).

EXAMPLE 7: IL-4 BIOASSAY PROTOCOL USING TF-1 (ATCC) CELLS.Reagents and Equipment Needed5 MTT Dye Solution:

MTT(3-[4,5-Dimethylthiazole-2-yl]) (Sigma catalog# M2128)

Working concentration: Dissolve 5 mg of anhydrous MTT in 200 ml PBS without Ca^{+2} , Mg^{+2} .

10 Sterile filter and store aliquoted at -20°C

Solubilization Solution:

For 1000 ml, combine 100 g SDS, 950 ml dH_2O , 50 ml Dimethyl Formamide,
15 and 850 μl concentrated HCl.

Filter sterilize with a $0.45\mu\text{m}$ filter unit.

Store at room temperature

TF-1 cell Growth Medium:

20

RPMI 1640, 10% FBS, Pen/Strep, 2mM L-glutamine

Other:

25 0.4% Trypan Blue Stain, sterile tubes for dilutions, sterile 96 well cell culture plates (Falcon #3072), hemacytometer, centrifuge, ELISA plate reader, multichannel pipet for 15, 25, 50 and 100 μl volume, sterile reagent reservoirs, sterile pipet tips, gloves.

Assay Protocol

A. Preparation of Assay plates

- 5 1. Prepare sterile 96 well tissue culture plates to contain 50µl of growth medium per well with various concentrations of IL-4 and 10nM IL-4 antagonist. This can be done by preparing a working dilution of IL-4 that is 4 times the highest concentration to be assayed. In separate tubes, do a two-fold serial dilution of the IL-4. Add 25µl of each dilution to one row
10 across the plate (i.e. row A gets highest concentration, row G gets lowest concentration). Add 25µl of growth medium without IL-4 to row H. Prepare the antagonists to be tested by making a stock that is 4 times the final concentration. Add 25µl to a triplicate set of IL-4 containing wells (columns 1,2,3, A through H). Be sure to include antagonist in row H.
15
2. As a positive control, leave one set with no antagonist. These wells will contain IL-4 and media only.
3. Incubate the plate for 1-2 hours at 37°C in a humidified 5% CO₂
20 incubator before preparing cells to be used for assay.

B. Preparation of Cells

- 25 4. Wash cells twice by centrifugation in assay medium free of growth factor.
5. Determine cell number and trypan blue viability and suspend cells to a final concentration of 8×10^5 /ml in assay medium.
- 30 6. Dispense 50µl of the cell suspension (40,000 cells) into all wells of the plates. Total volume should now be 100µl/well.

7. Incubate the plate at 37°C for 68 hours in a humidified 5% CO₂ incubator.

C. Color Development

5

8. After incubating for 68 hours, add 15μl of the MTT dye solution to each well.

9. Incubate the plate at 37°C for 4 hours in a humidified 5% CO₂ incubator.

10

10. After 4 hours, add 100μl of the solubilization solution to each well. Allow the plate to stand overnight in a sealed container to completely solubilize the formazan crystals.

15 11. Record the absorbance at 570/650nm.

RESULTS

Figure 27 shows that an IL-4 trap designated 4SC375, which is a fusion polypeptide of IL-2Rγ-scb-IL4Rα-FcΔC1, is several orders of magnitude better as an IL-4 antagonist than IL4RαFcΔC1 alone in the TF1 cell bioassay.

Figure 28 shows that the IL-4 trap designated 4SC375 shows antagonistic activity in the TF1 cell bioassay equivalent to an IL-4 trap designated 4SC424 which is a fusion polypeptide of IL-2Rγ-IL4Rα-FcΔC1 having the IL-2Rγ component flush with the IL-4Rα component.

EXAMPLE 8: IL-6 BIOASSAY PROTOCOL USING XG-1 CELLS

30 Reagents and Equipment Needed

MTT Dye Solution:

MTT(3-[4,5-Dimethylthiazole-2-yl]) (Sigma catalog# M2128)

Working concentration: Dissolve 5 mg of anhydrous MTT in 200 ml PBS

5 without Ca^{+2} , Mg^{+2} .

Sterile filter and store aliquoted at -20°C

Solubilization Solution:

10 For 1000 ml, combine 100 g SDS, 950 ml dH_2O , 50 ml Dimethyl Formamide, and 850 μl concentrated HCl.

Filter sterilize with at $0.45\mu\text{m}$ filter unit.

Store at room temperature

15 Assay Medium:

RPMI 1640, 10%FBS, Pen/Strep, 2mM L-glutamine, $50\mu\text{M}$ mercapto-ethanol.

20 Other:

0.4% Trypan Blue Stain, sterile tubes for dilutions, sterile 96 well cell culture plates (Falcon#3072), hemacytometer, centrifuge, ELISA plate reader, multichannel pipet for 15, 25, 50 and $100\mu\text{l}$ volume, sterile reagent

25 reservoirs, sterile pipet tips, gloves.

Assay ProtocolA. Preparation of Assay plates

30

1. Prepare sterile 96 well tissue culture plates to contain $50\mu\text{l}$ of growth medium per well with various concentrations of IL-6 and 10nM IL-6 antagonist. This can be done by preparing a working dilution of IL-6 that is

- 4 times the highest concentration to be assayed. In separate tubes, do a two-fold serial dilution of the IL-6. Add 25 μ l of each dilution to one row across the plate (i.e. row A gets highest concentration, row G gets lowest concentration). Add 25 μ l of growth medium without IL-6 to row H.
- 5 Prepare the antagonists to be tested by making a stock that is 4 times the final concentration. Add 25 μ l to a triplicate set of IL-6 containing wells (columns 1,2,3, A through H). Be sure to include antagonist in row H. A typical IL-6 titration starts at 200ng/ml down to 3.1ng/ml.
- 10 2. As a positive control, leave one set with no antagonist. These wells contain IL-6 and media in place of antagonist.
3. Incubate the plate 1-2 hours at 37°C in a humidified 5% CO₂ incubator before preparing cells to be used for assay.

15

B. Preparation of Cells

4. Wash cells twice by centrifugation (5 min at 1000RPM) in assay medium free of growth factor.
- 20 5. Determine cell number and trypan blue viability and suspend cells to a final concentration of 8×10^5 /ml in assay medium.
6. Dispense 50 μ l of the cell suspension (40000 cells) into all wells of the
- 25 plates. Total volume should now be 100 μ l/well.
7. Incubate the plate at 37°C for 68 hours in a humidified 5% CO₂ incubator.

C. Color Development

8. At 68 hours add 15 μ l of the dye solution to each well.

9. Incubate the plate at 37°C for 4 hours in a humidified 5% CO₂ incubator.
10. After 4 hours, add 100µl of the solubilization solution to each well.
Allow the plate to stand overnight in a sealed container to completely
5 solubilize the formazan crystals.
11. Record the absorbance at 570/650nm.

RESULTS

10

Figure 29 shows that the IL6 trap (6SC412 IL6R-scb-gpx-FcΔC1) described in Figure 24A - Figure 24F is a better antagonist of IL-6 in the XG1 bioassay than the neutralizing monoclonal antibody to human IL-6 - BE8.

15 EXAMPLE 9: MRC5 BIOASSAY FOR IL1 TRAPS

MRC5 human lung fibroblast cells respond to IL-1 by secreting IL-6 and thus were utilized to assay the ability of IL-1 traps to block the IL-1-dependent production of IL-6. IL1 Trap 1SC569 (Figure 26A - Figure 26E)
20 was tested against IL-1-RI.Fc which is the extracellular domain of the IL-1 Type I receptor fused to an Fc domain.

MRC5 cells are suspended at 1×10^5 cells per ml in medium and 0.1 ml of cells are plated (10,000 cells per well) into the wells of a 96 well tissue
25 culture plate. Plates are incubated for 24 hours at 37°C in a humidified 5% CO₂ incubator.

IL-1 trap and recombinant human IL-1 at varying doses are pre-incubated in a 96 well tissue culture dish and incubated for 2 hours at 37°C. 0.1 ml of
30 this mixture is then added to the 96 well plate containing the MRC5 cells such that the final concentration of IL-1 Trap is 10nM and the final

concentrations of the IL-1 ranges from 2.4 pM to 5nM. Control wells contain trap alone or nothing.

Plates are then incubated at 37°C for 24 hours in a humidified 5% CO₂ incubator. Supernatant is collected and assayed for levels of IL-6 using R&D Systems Quantikine Immunoassay Kit according to the manufacturer's instructions.

RESULTS

10

Figure 30 shows that the trap 569 (Figure 26A - Figure 26E) is able to antagonize the effects of IL-1 and block the IL-6 production from MRC 5 cells upon treatment with IL-1. At a concentration of 10nM, the trap 569 is able to block the production of IL-6 up to an IL-1 concentration of 3nM. In contrast, the IL-1RI.Fc is a much poorer antagonist of IL-1. It is only able to block the effects of IL-1 up to about 10-20 pM. Thus, the trap 569 is approximately 100x better at blocking IL-1 than IL1RI.Fc.

EXAMPLE 10 - CONSTRUCTION OF IL-13/IL-4 SINGLE CHAIN TRAPS

20

1. To create the IL-13/IL-4 dual trap designated IL-4R α .IL-13R α 1.Fc, the human IL-4R α extracellular domain (corresponding to nucleotides #1-693 of Figure 31A - Figure 31G) and the human IL-13R α 1 extracellular domain (corresponding to nucleotides #700-1665 of Figure 31A - Figure 31G) were amplified by standard PCR techniques and ligated into an expression vector pMT21 which contained the human Fc sequence (corresponding to nucleotides #1671-2355 of Figure 31A - Figure 31G), thus creating a fusion protein consisting of the IL-4R α , IL-13R α 1, and the hinge, CH2 and CH3 region of human IgG1 from the N to C terminus. In addition, a two amino acid linker (corresponding to nucleotides #694-699 of Figure 31A - Figure 31G) with the amino acid sequence SerGly was constructed in frame

- between the IL-4R α and the IL-13R α 1 and a two amino acid linker (corresponding to nucleotides #1666-1671 of Figure 31A - Figure 31G) with the amino acid sequence ThrGly was constructed in frame between the IL-13R α 1 and the Fc portion. All sequences were sequence-verified by
- 5 standard techniques. The IL-4R α .IL-13R α 1.Fc coding sequence was then subcloned into the expression vector pCDNA3.1 (Stratagene) using standard molecular biology techniques.
2. To create the IL-13/IL-4 dual trap designated IL-13R α 1.IL-4R α .Fc, the IL-
- 10 13R α 1 extracellular domain (corresponding to nucleotides #1-1029 of Figure 32A - Figure 32G) and the human IL-4R α (corresponding to nucleotides # 1060-1692 of Figure 32A - Figure 32G) were amplified by standard PCR techniques and ligated into the expression vector pJFE14, which contains the human Fc sequence (corresponding to nucleotides
- 15 #1699-2382 of Figure 32A - Figure 32G) to create a fusion protein consisting of the IL-13R α 1, IL-4R α , and the hinge, CH2 and CH3 region of human IgG1 from the N to C terminus. In addition, a ten amino acid linker with the amino acid sequence GlyAlaProSerGlyGlyGlyGlyArgPro (corresponding to nucleotide #1030-1059 of Figure 32A - Figure 32G) was
- 20 constructed in frame between the IL-13R α 1 and the IL-4R α and a two amino acid linker (corresponding to nucleotides #1693-1698 of Figure 32A - Figure 32G) with the amino acid sequence SerGly was constructed in frame between IL-4R α and the Fc portion. All sequences were sequence-verified using standard techniques. The coding sequence of IL-13R α 1.IL-4R α .Fc
- 25 was then subcloned into the expression vector pCDNA3.1 (Stratagene) using standard molecular biology techniques.

EXAMPLE 11: EXPRESSION OF IL-4R α .IL-13R α 1.Fc AND IL-13R α 1.IL-4R α .Fc

Large scale (1L) cultures of the pCAE801 (the DNA vector construct encoding IL-4R α .IL-13R α 1.Fc) and pCAE802 (the DNA plasmid construct encoding IL-13R α 1.IL-4R α .Fc) in DH10B cells were grown overnight in LB + ampicillin and the plasmid DNA was extracted using a Qiagen Endofree
5 Mega Kit following the manufacturer's protocol. The concentration of the purified plasmid DNA was determined in a UV spectrophotometer and fluorometer. The plasmid DNA was also verified by digestion of aliquots with BbsI, XmnI and NcoI restriction enzymes. All restriction enzyme digest fragments corresponded to the predicted sizes in a 1% agarose gel.

10

Forty 15 cm petri plates were seeded with CHO-K1/E1A cells at a density of 4×10^6 cells/plate. Plating media was Gibco Ham's F-12 w/10% Hyclone Fetal Bovine Serum (FBS) + penicillin/streptomycin and supplemented with glutamine. The following day each plate was transfected with 6 μ g of
15 pCAE801, or pCAE802, using Gibco Optimem and Gibco Lipofectamine in 12 ml volume, following the manufacturer's protocol. Four hours after adding the transfection mix to the cells 12 ml/plate of Optimem w/ 10% FBS was added. Plates were incubated at 37°C in a 5% CO₂ incubator overnight. The following day the media was removed from each plate
20 and 25 ml expression media (Gibco CHO-S-SFM II w/ glutamine + 1mM sodium butyrate) was added. The plates were incubated at 37°C for 3 days.

After 3 days of incubation the media was removed from each plate and centrifuged at 400 rpm in a swinging bucket rotor to pellet cells. The
25 supernatant was decanted into sterile 1L bottles and expressed protein was purified as described *infra*.

EXAMPLE 12: PURIFICATION OF IL-4R α .IL-13R α 1.Fc AND IL-13R α 1.IL-4R α .Fc PROTEIN FROM CULTURE MEDIA

30

1. Purification of IL-4R α .IL-13R α 1.Fc.

Human IL-4R α .IL-13R α 1.Fc was transiently expressed in CHO cells and supernatants were harvested from plate transfections as described *supra*.

5 Expression of the secreted protein was determined by a sandwich ELISA using goat anti-hIgG (γ chain specific; Sigma 1-3382) and goat anti-hIgG (Fc specific)-FITC conjugate (Sigma F9512) capture and report antibodies, respectively. The yield ranged from 5.8 to 9.2 mg (average of 7.5 mg) per liter of conditioned media. CompleteTM protease inhibitor tablets (Roche

10 Diagnostics Corp.) were dissolved into the media (1 tablet/L). The conditioned media was sterile filtered (0.22 μ m pore size) prior to loading onto a pre-equilibrated, 5 mL HiTrap[®] Protein A affinity column (Amersham Pharmacia Biotech) in Dulbecco's PBS buffer (Life Technologies), pH 7.4 at 4°C. The flow rate was ~1-2 mL/min. The

15 column was extensively washed with PBS buffer to remove nonspecifically bound proteins from the column. IL-4R α .IL-13R α 1.Fc was eluted using 20 mM sodium citrate, 150 mM NaCl, pH 3.5. The eluate was immediately neutralized by titrating with 1 M Tris-OH. The fractions containing protein were pooled and immediately dialyzed in PBS buffer,

20 pH 7.4 at 4°C. The recovery from Protein A purification was 6.8 mg (73%). IL-4R α .IL-13R α 1.Fc was further purified by size exclusion chromatography using a superose 6 column (25 mL bed volume; Amersham Pharmacia Biotech) pre-equilibrated in PBS, 5% v/v glycerol, pH 7.4 at ambient temperature. The flow rate was 0.5 mL/min. Protein fractions were

25 assessed from a Coomassie stained non-reduced and reduced SDS-PAGE (Novex NuPAGE 4-12% Bis-Tris gels). Fractions were conservatively pooled to reduce the amount of aggregated protein. The overall yield was 51% (4.4 mg) with a purity of 97% as judged by SDS-PAGE. Purified IL-4R α .IL-13R α 1.Fc was analyzed by non-reduced and reduced SDS-PAGE (4-

30 12% Bis-Tris), analytical size exclusion chromatography (Tosohaas

TSKG4000SWXL), N-terminal sequencing, and immunoblotting with goat anti-hIgG-HRP conjugate (Promega W403B), and also mouse monoclonal anti-hIL-4R (R&D MAB230) followed by anti-mIgG-HRP conjugate (Promega W402B) as the secondary antibody.

5

2. Purification of IL-13R α 1.IL-4R α .Fc

Human IL-13R α 1.IL-4R α .Fc was transiently expressed in CHO cells and supernatants were harvested from plate transfections as described *supra*.

- 10 Expression of the secreted protein was determined by a sandwich ELISA using goat anti-hIgG (γ chain specific; Sigma 1-3382) and goat anti-hIgG (Fc specific)-FITC conjugate (Sigma F9512) capture and report antibodies, respectively. The yield was 8.8 mg per liter of conditioned media. Complete[™] protease inhibitor tablets (Roche Diagnostics Corp.) were
- 15 dissolved into the media (1 tablet/L). The conditioned media was sterile filtered (0.22 μ m pore size) prior to loading onto a pre-equilibrated, 5 mL HiTrap[®] Protein A affinity column (Amersham Pharmacia Biotech) in Dulbecco's PBS buffer (Life Technologies), pH 7.4 at 4°C. The flow rate was ~1-2 mL/min. The column was extensively washed with PBS buffer to
- 20 remove nonspecifically bound proteins from the column. IL-13R α 1.IL-4R α .Fc was eluted using 20 mM sodium citrate, 150 mM NaCl, pH 3.5. The eluate was immediately neutralized by titrating with 1 M Tris-OH. The fractions containing protein were pooled and immediately dialyzed in PBS buffer, pH 7.4 at 4 °C. The recovery from Protein A purification was 3.8 mg
- 25 (43%). IL-13R α 1.IL-4R α .Fc was further purified by size exclusion chromatography using a superose 6 column (25 mL bed volume; Amersham Pharmacia Biotech) pre-equilibrated in PBS, 5% v/v glycerol, pH 7.4 at ambient temperature. The flow rate was 0.5 mL/min. Protein fractions were assessed from a Coomassie stained non-reduced and
- 30 reduced SDS-PAGE (Novex NuPAGE 4-12% Bis-Tris gels). Fractions were

conservatively pooled to reduce the amount of aggregated protein. The overall yield was 17% (1.5 mg) with a purity of 95% as judged by SDS-PAGE. Purified IL-13R α 1.IL-4R α .Fc was analyzed by non-reduced and reduced SDS-PAGE (4-12% Bis-Tris), analytical size exclusion

- 5 chromatography (Tosohaas TSKG4000SWXL), N-terminal sequencing, and immunoblotting with goat anti-hIgG-HRP conjugate (Promega W403B), and also mouse monoclonal anti-hIL-4R α (R&D MAB230) followed by anti-mIgG-HRP conjugate (Promega W402B) as the secondary antibody.

10 EXAMPLE 13: BLOCKING OF IL-4 AND IL-13 BY IL-4R α .IL-13R α 1.Fc AND IL-13R α 1.IL-4R α .Fc

Materials and Methods

- 15 TF1 Bioassay. TF1 cells were maintained in growth media (10ng/ml GM-CSF, RPMI 1640, 10% FBS, L-glutamine, Penicillin, Streptomycin). For the bioassay, cells were washed 2 times in assay media (as above but without GM-CSF) and then plated at 2×10^5 cells in 50 μ l of assay media. The purified IL-4R α .IL-13R α 1.Fc and IL-13R α 1.IL-4R α .Fc proteins were diluted
- 20 into assay media at a concentration of 40nM. 25 μ l of each of the traps was added to the cells. Either IL-13 or IL-4 were diluted to 40nM in assay media and then 2-fold dilution series in assay media were made. 25 μ l of either IL-13 or IL-4 was then added to the wells containing the cells and the traps. Cells were then incubated at 37°C, 5% CO₂ for ~70 hrs. The extent of TF1
- 25 cell proliferation was measured by the MTS assay according to the manufacturer's protocol (Promega, Inc.).

RESULTS

- 30 The ability of the IL-4R α .IL-13R α 1.Fc and IL-13R α 1.IL-4R α .Fc traps to block both human IL-13 and human IL-4 activity was measured in the TF1

bioassay described *supra*. IL-13 stimulates proliferation of TF1 cells, with half-maximal growth at a concentration of 0.2nM. Addition of either IL-4R α .IL-13R α 1.Fc or IL-13R α 1.IL-4R α .Fc trap at a concentration of 10nM blocks IL-13-induced growth up to ~2nM (Figure 33). At an IL-13 concentration of ~4-5 nM the growth of TF1 cells is inhibited by 50%. TF1 cells are more sensitive to IL-4, which stimulates their proliferation with half-maximal growth at ~0.02nM. Addition of either IL-4R α .IL-13R α 1.Fc or IL-13R α 1.IL-4R α .Fc at a concentration of 10nM blocks IL-4-induced growth up to ~1nM (Figure 34). At an IL-4 concentration of ~3-4 nM the growth of TF1 cells is inhibited by 50%. These results show that both IL-4R α .IL-13R α 1.Fc and IL-13R α 1.IL-4R α .Fc can block the ability of both IL-13 and IL-4 to stimulate cellular responses.

EXAMPLE 14: BLOCKING OF INJECTED IL-1 BY IL-1 TRAP *IN VIVO*

IL-1 is a pro-inflammatory cytokine. Systemic administration of IL-1 has been shown to elicit acute responses in animals, including transient hyperglycemia, hypoinsulinemia, fever, anorexia, and increased serum levels of interleukin-6 (IL-6) (Reimers, 1998). Since mice are responsive to both murine and human IL-1, human IL-1 can be used and *in vivo* binding effects of human specific IL-1 antagonists can be evaluated. This acute mouse model was used to determine the ability of a human IL-1 trap to antagonize the *in vivo* effects of exogenously administered human IL-1. This provides a rapid indication of *in vivo* efficacy of the human IL-1 trap and can be used as an assay to help molecule selection.

Experimental Design:

Mice were given subcutaneous injections of human IL-1 (0.3 μ g/kg). Twenty-four hours prior to human IL-1 injection, the animals were pre-treated with either vehicle or 150-fold molar excess of human IL-1 trap (0.54 mg/kg). Two hours prior to sacrifice (26 hrs), the mice were given a

second injection of human IL-1 (0.3 µg/kg). Blood samples were collected at various time points and sera were assayed for IL-6 levels.

RESULTS

5

Exogenous administration of human IL-1 resulted a dramatic induction of serum IL-6 levels. At 150-fold molar excess, the human IL-1 trap completely blocked the IL-6 increase (Figure 35). Furthermore, the effects of the human IL-1 trap persisted for at least another 24 hours, preventing an IL-6 increase even when IL-1 was re-administered (Figure 35). Such long-lasting efficacy suggests that daily injection of an IL-1 trap may not be necessary for chronic applications.

EXAMPLE 15: EVALUATING THE ABILITY OF AN IL-4 TRAP TO BLOCK THE PHYSIOLOGICAL RESPONSES TO HUMAN IL-4 IN CYNOMOLOGUS MONKEYS.

Systemic administration of human IL-4 elicits systemic responses in Cynomologus monkeys (Gundel et al., 1996). Thus, the effectiveness of the IL-4 trap in blocking human IL-4 can be demonstrated by measuring these responses.

Experimental Design:

The experiment consisted of 3 parts: human IL-4 + vehicle (part 1), human IL-4 + IL-4 Trap (part 2), and human IL-4 + vehicle (part 3). Human IL-4 (25 µg/kg) was injected subcutaneously twice daily for 4 days and IL-4 Trap (8 mg/kg) and vehicle were given intravenously daily for 5 days, beginning 1 day prior to human IL-4 administration. Whole blood was collected daily for flow cytometry analysis for CD16 and plasma was obtained to assay for the cytokine monocyte chemotactic protein 1 (MCP-1).

CD16 and MCP-1 are markers of IL-4-mediated inflammation in both humans and monkeys.

RESULTS

5

In the presence of human IL-4, MCP-1 increased 2.5-fold and was significantly blocked by the IL-4 Trap (Figure 36A). Similarly, the decrease in the percent of CD16 positive lymphocytes in peripheral blood was attenuated by the IL-4 trap (Figure 36B). After a rest period, the monkeys
10 were re-injected with human IL-4 and the responsiveness of the animals to human IL-4 was re-confirmed (Figures 36A and 36B), suggesting that inhibition of the MCP-1 and CD 16 responses is specifically mediated by the IL-4 trap.

15 EXAMPLE 16: THE EFFECTS OF IL-4 TRAP ON IL-4-INDUCED IgE SECRETION.

It has been shown that injection of anti-mouse IgD antibody stimulates an IL-4-mediated IgE increase in normal mice. This model has been widely
20 used to evaluate IL-4 antagonists, such as soluble IL-4 receptor and anti-IL-4 monoclonal antibodies (Sato et al., 1993). We decided to use this model to evaluate the ability of the IL-4 trap to block IL-4-mediated increases of IgE.

25 Experimental design:

BALB/C mice injected with anti-mouse IgD (100µl/mouse, s.c.) were randomly divided into 3 groups. Each received (on days 3-5) either vehicle, murine IL-4 trap (1 mg/kg, s.c.), or a monoclonal antibody to
30 mouse IL-4 (1 mg/kg, s.c.). Serum was collected at various time points and assayed for IgE levels.

RESULTS

Treatment with the murine IL-4 trap or the mouse IL-4 antibody both significantly antagonized the IL-4-mediated IgE increase in this mouse
5 model (Figure 37). This suggests that the murine IL-4 trap binds murine IL-4 and antagonizes physiological responses elicited by endogenous IL-4 *in vivo*.

The present invention is not to be limited in scope by the specific
10 embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

15

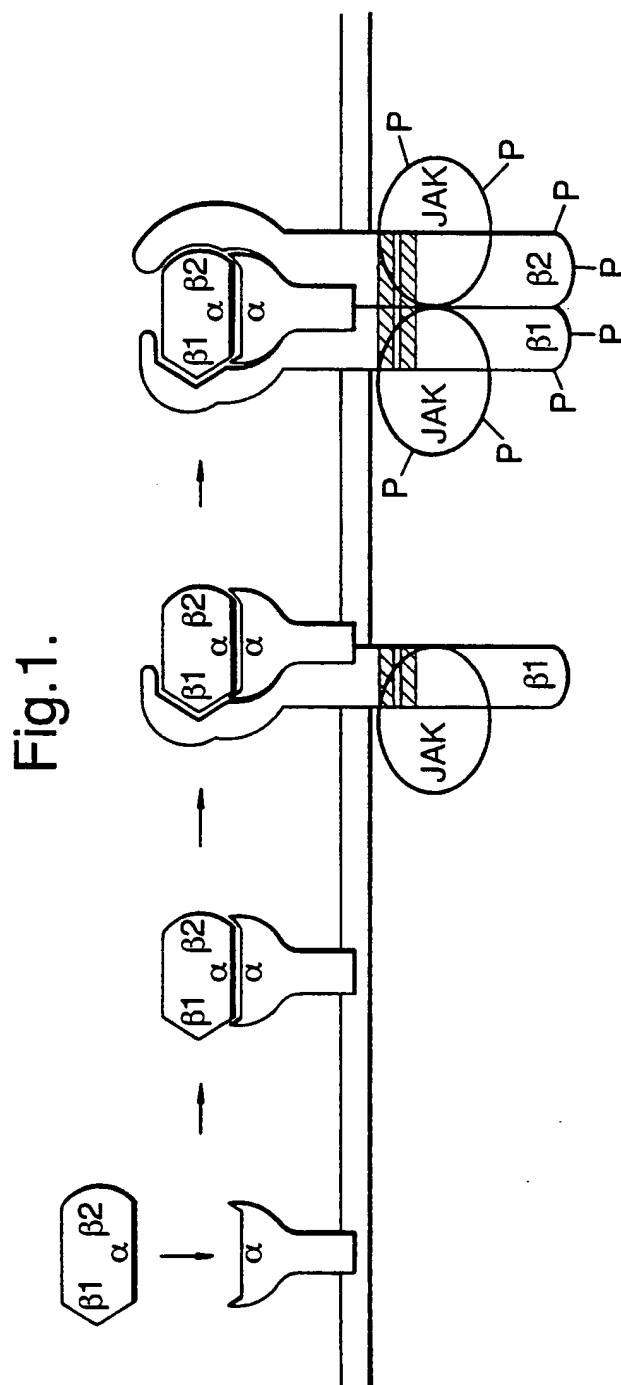
WE CLAIM:

1. An isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex
5 comprising:
 - a) a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the specificity determining component of the cytokine's receptor;
 - 10 b) a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the signal transducing component of the cytokine's receptor; and
 - c) a nucleotide sequence encoding a third fusion polypeptide
15 component comprising the amino acid sequence of a multimerizing component.
2. The nucleic acid molecule of claim 1, wherein the nucleotide sequence encoding the first component is upstream of the nucleotide
20 sequence encoding the second component.
3. The nucleic acid molecule of claim 1, wherein the nucleotide sequence encoding the first component is downstream of the nucleotide
25 sequence encoding the second component.
4. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the hematopoietin family of cytokines selected from the group consisting of interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-9,
30 interleukin-11, interleukin-13, interleukin-15, granulocyte macrophage colony stimulating factor, oncostatin M, and leukemia inhibitory factor and cardiotrophin-1

5. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the interferon family of cytokines selected from the group consisting of IFN-gamma, IFN-alpha, and IFN-beta.
- 5 6. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the immunoglobulin superfamily of cytokines selected from the group consisting of B7.1 (CD80) and B7.2 (B70).
- 10 7. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the TNF family of cytokines selected from the group consisting of TNF-alpha, TNF-beta, LT-beta, CD40 ligand, Fas ligand, CD 27 ligand, CD 30 ligand, and 4-1BBL.
- 15 8. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the TGF- β /BMP family selected from the group consisting of TGF- β 1, TGF- β 2, TGF- β 3, BMP-2, BMP-3a, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8a, BMP-8b, BMP-9, BMP-10, 20 BMP-11, BMP-15, BMP-16, endometrial bleeding associated factor (EBAF), growth differentiation factor-1 (GDF-1), GDF-2, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-12, GDF-14, mullerian inhibiting substance (MIS), activin-1, activin-2, activin-3, activin-4, and activin-5.
- 25 9. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a cytokine selected from the group consisting of interleukin-1, interleukin-10, interleukin-12, interleukin-14, interleukin-18 and MIF.
- 30 10. The isolated nucleic acid molecule of claim 1, wherein the multimerizing component comprises an immunoglobulin derived domain.

11. The isolated nucleic acid molecule of claim 10, wherein the immunoglobulin derived domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.
- 5 12. A fusion polypeptide encoded by the isolated nucleic acid molecule of claim 1.
13. A composition capable of binding a cytokine to form a nonfunctional complex comprising a multimer of the fusion polypeptide of claim 12.
- 10 14. The composition of claim 13, wherein the multimer is a dimer.
- 15 15. A vector which comprises the nucleic acid molecule of claim 1.
16. An expression vector comprising a nucleic acid molecule of claim 1, wherein the nucleic acid molecule is operatively linked to an expression control sequence.
- 20 17. A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 16, in a suitable host cell.
18. The host-vector system of claim 17, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell, or mammalian cell.
- 25 19. The host-vector system of claim 17, wherein the suitable host cell is E. coli.
- 30 20. The host-vector system of claim 17, wherein the suitable host cell is a COS cell.

21. The host-vector system of claim 17, wherein the suitable host cell is a CHO cell.
22. The host-vector system of claim 17, wherein the suitable host cell is
5 a 293 cell.
23. The host-vector system of claim 17, wherein the suitable host cell is a BHK cell.
- 10 24. The host-vector system of claim 17, wherein the suitable host cell is a NS0 cell.
25. A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claim 17, under conditions
15 permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.



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Fig.2.

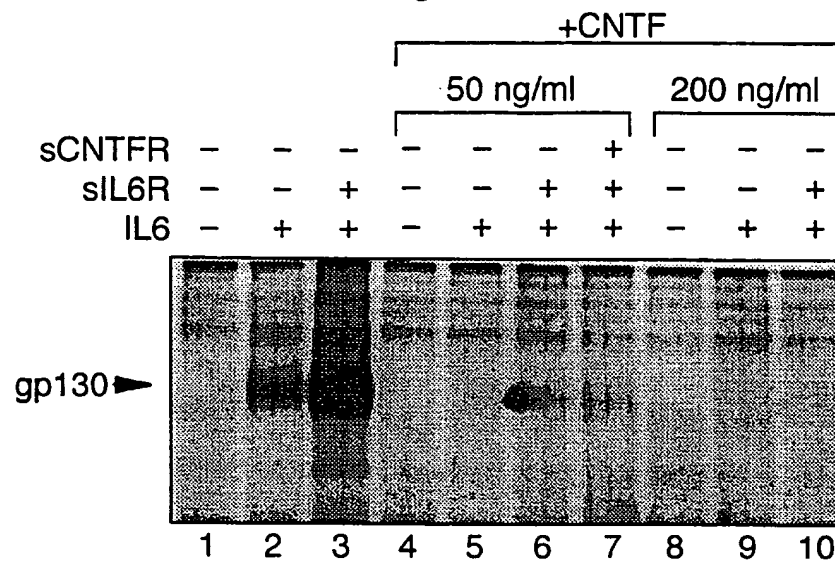
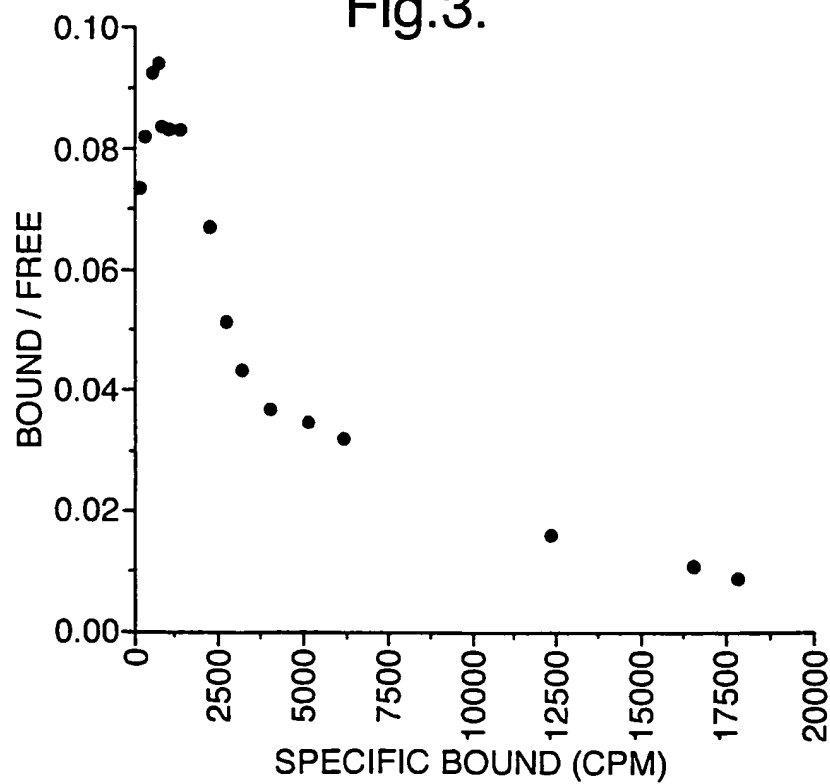


Fig.3.



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Fig.4.

Amino acid sequence of human gp130-Fc-His6

Sequence Range: 1 to 861

10	20	30	40	50	60
*	*	*	*	*	*
MVTLQTWVQALFIFLT	TES	TGELLDP	CGYISP	ESPVVQ	L
70	80	90	100	110	120
*	*	*	*	*	*
NANYIVWKT	NHFTIPKEQYT	IINRTASSVTFTDIASL	NIQ	LTCN	ILTFGQLEQNVYGITI
130	140	150	160	170	180
*	*	*	*	*	*
ISGLPPEKPKNL	SCIVNEGK	KMRCEWDGGRETHLETN	FTL	KSEWATHK	FADCKAKRDTPT
190	200	210	220	230	240
*	*	*	*	*	*
SCTVDYSTVYFVNIEV	WVEA	ENALGKV	TSDHINFDPVYKV	KPNPPHNLSVINSEEL	SSIL
250	260	270	280	290	300
*	*	*	*	*	*
KLWTNPSIKSVIILKY	NIQ	YRTKDASTWSQIPPEDT	AST	RSSFTVQDLKPFTEYV	FRIR
310	320	330	340	350	360
*	*	*	*	*	*
CMKEDGKGYWSDWSEE	ASGI	TYEDRPSKAPSF	WYKIDPSH	TQGYRTVQ	LVWKTLP
370	380	390	400	410	420
*	*	*	*	*	*
GKILDYEVTLTRWKSH	LQNY	TVNATKLTVNLTND	RYLATL	TVRN	LVGKS
430	440	450	460	470	480
*	*	*	*	*	*
FQATHPVMDLKAFPKD	NMLW	VEWTTPRESVKYI	LEWCVL	SDKAPCITDWQ	QEDGTVHRT
490	500	510	520	530	540
*	*	*	*	*	*
YLRGNLAESKCYLITV	TPVY	ADGPGSPESIKAYL	KQAPPS	KGPTV	RTKKVGKNEAVLEWD
550	560	570	580	590	600
*	*	*	*	*	*
QLPVDVQNGFIRNYTIF	YRT	IIGNETAVNV	DSSHTEY	TL	SLTSDTLYMVRMAAYTDEGG
610	620	630	640	650	660
*	*	*	*	*	*
KDGPEFTFTTPKFAQGE	IES	GEPKSCDKTHTCP	PCPAPEL	LGGPSV	FLFPPKPKDTLMIS
670	680	690	700	710	720
*	*	*	*	*	*
RTPEVTCVVVDVSHED	PEVK	FNWYVDGVEVHNA	TKPREE	OYNSTYRV	VSVLTVLHODWL
730	740	750	760	770	780
*	*	*	*	*	*

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Fig.4 (Cont).

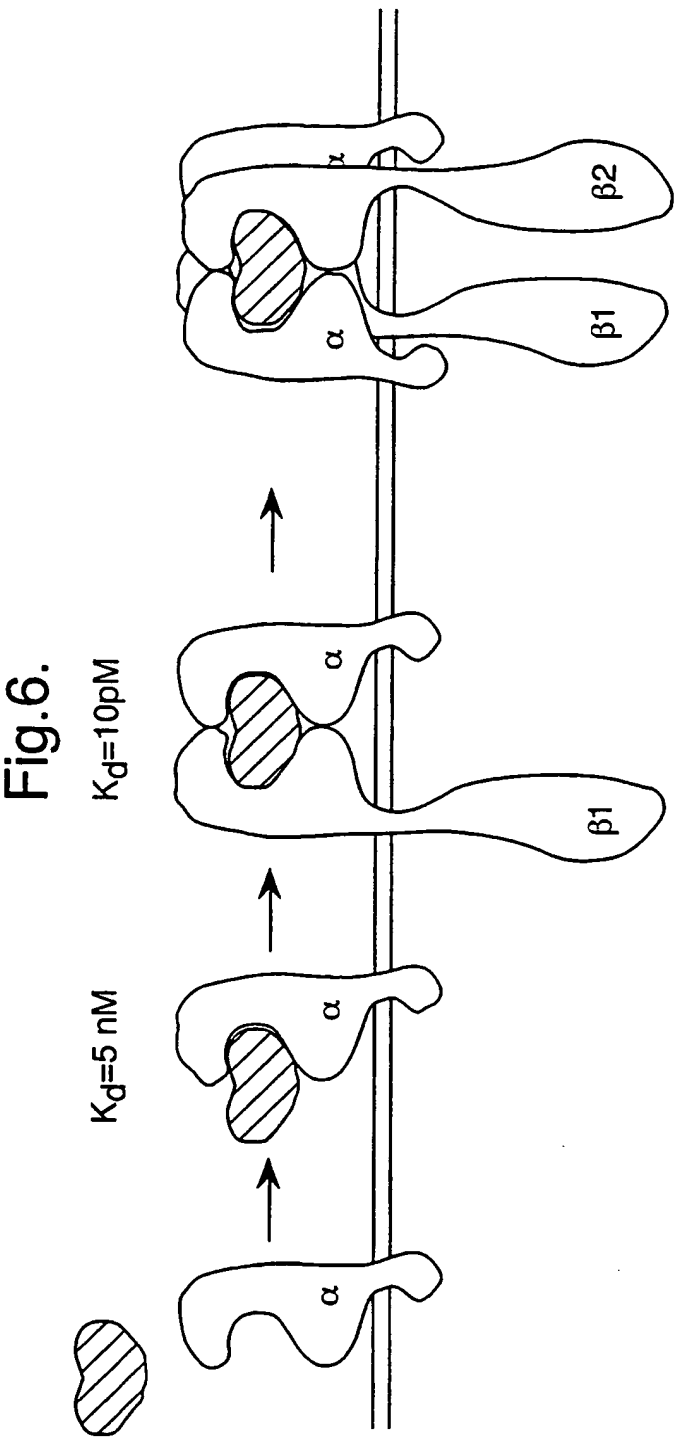
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 850 860
 * *
HYTOKSLSLSPGKHHHHH•

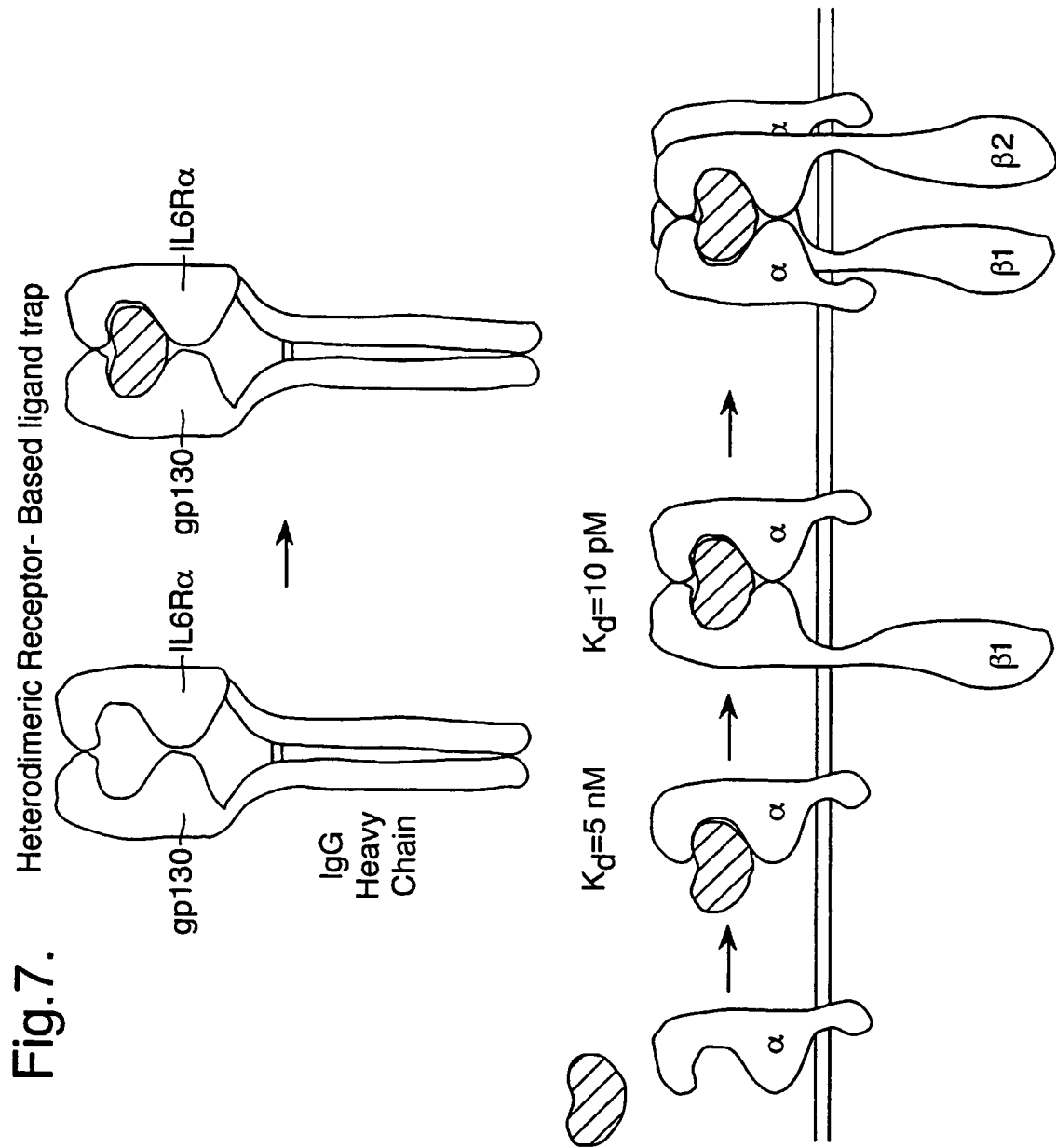
Fig.5.

The amino acid sequence of human IL-6R α -Fc

Sequence Range: 1 to 594

10	20	30	40	50	60
*	*	*	*	*	*
MVAVGCALLAALLAAPGAAL	APRRCPAQEVARGVLTSLPG	DSVTLTCPGVEPEDNATVHW			
70	80	90	100	110	120
*	*	*	*	*	*
VLRKPAAGSHPSRWAGMGRR	LLLRSVQLHDSGNYSCYRAG	RPAGTVHLLVDVPPEEPQLS			
130	140	150	160	170	180
*	*	*	*	*	*
CFRKSPLSNVVCEWGPRSTP	SLTTKAVLLVRKFQNSPAED	FQEPQCYSQESQKFSCQLAV			
190	200	210	220	230	240
*	*	*	*	*	*
PEGDSSFYIVSMCVASSVGS	KFSKTQTFQCGILQPDPPA	NITVTAVARNPRWLSVTWQD			
250	260	270	280	290	300
*	*	*	*	*	*
PHSWNSSFYRLRFELRYRAE	RSKTFTTWMVKDLQHHCVIH	DAWSGLRHVVQLRAQEEFGQ			
310	320	330	340	350	360
*	*	*	*	*	*
GEWSEWSPEAMGTPWTESRS	PPAENEVSTPMQALTTNKDD	DNILFRDSANATSLPVQDAG			
370	380	390	400	410	420
*† †	*	*	*	*	*
EPKSCDKTHTCPPCPAPELL	GGPSVFLFPPKPKDTLMISR	TPEVTCVVVDVSHEDPEVKF			
430	440	450	460	470	480
*	*	*	*	*	*
NWYVDGVEVHNAKTKPREEO	YNSTYRVVSVLTVLHODWLN	GKEYKCKVSNKALPAPIEKT			
490	500	510	520	530	540
*	*	*	*	*	*
ISKAKGOPREPOVYTLPPSR	DELTKNOVSLTCLVKGFYPS	DIAVEWESNGOPENNYKTTT			
550	560	570	580	590	
*	*	*	*	*	
PVLDSGDSFFLYSKLTVDKS	RWOOGNVFSCSVMEALHNH	YTOKSLSLSPGK•			

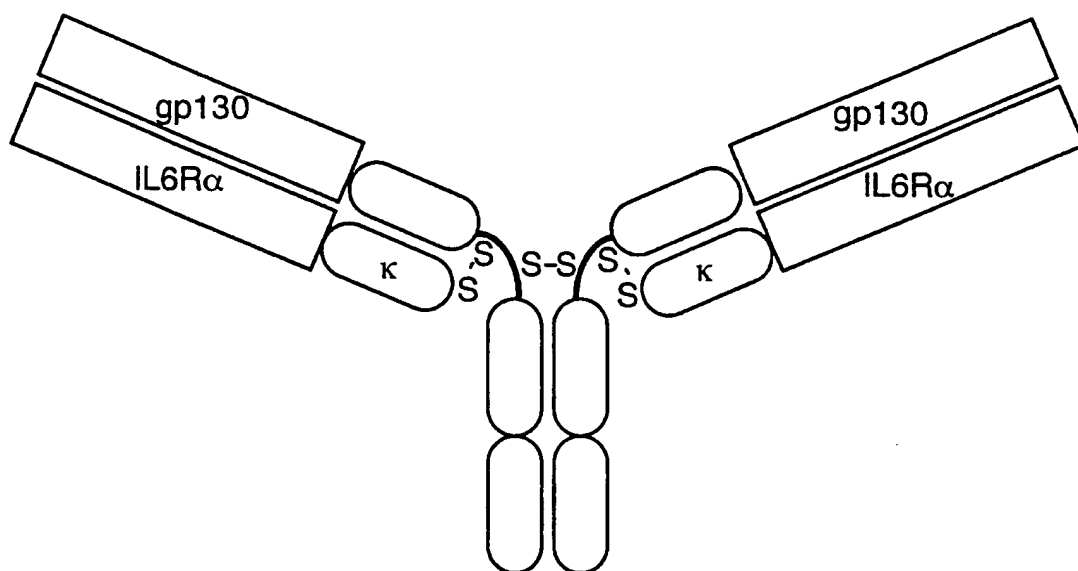




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Fig.8.

Immunoglobulin Heavy/Light Chain receptor Fusions



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Fig.9.

Amino acid sequence of gp130-Cy1

Sequence Range: 1 to 952

10	20	30	40	50	60
*	*	*	*	*	*
MVTLQTWVVQALFIFLT	TES	TGELLDPCGYISPES	PVVQL	HSNFTAVCVLKEK	CMDYFHV
70	80	90	100	110	120
*	*	*	*	*	*
NANYIVWKTNHFTIPKE	QYT	IINRTASSVTFTDI	ASLNIQ	LTCNILTFGQLEQ	NVYGITI
130	140	150	160	170	180
*	*	*	*	*	*
ISGLPPEKPKNLSCIV	NEGK	KMRCEWDGGRETH	LETNFTL	KSEWATHKFADCK	AKRDTPT
190	200	210	220	230	240
*	*	*	*	*	*
SCTVDYSTVYFVNIEV	WVEA	ENALGKVTSDHIN	FDPVYKV	KPNPPHNLSVIN	SEELSSIL
250	260	270	280	290	300
*	*	*	*	*	*
KLTWTNPSIKSVIILK	YNIQ	YRTKDASTWSQIP	PEDTAST	RSSFTVQDLKPF	TEYVFRIR
310	320	330	340	350	360
*	*	*	*	*	*
CMKEDGKGYSDWSEE	ASGI	TYEDRPSKAPSF	WYKIDPSH	TQGYRTVQLV	WKTLPPFEAN
370	380	390	400	410	420
*	*	*	*	*	*
GKILDYEVTLTRWK	SHLQNY	TVNATKLTVNLT	NDRYLATL	TVRNLVGKSD	AAVLTI
430	440	450	460	470	480
*	*	*	*	*	*
FQATHPVMDLKAF	PKDNMLW	VEWTTPRESV	KKYILEWCVL	SDKAPCITD	WQQEDGTVHRT
490	500	510	520	530	540
*	*	*	*	*	*
YLRGNLAESKCYL	ITVTPVY	ADGPGSPES	IKAYLKQAPPS	KGPTVRTK	KVGKNEAVLEWD
550	560	570	580	590	600
*	*	*	*	*	*
QLPVDVQNGFIR	NYTIFYRT	IIGNETAVN	VDSSHTEY	TL	SLTSDTLYM
610	620	630	640	650	660
*	*	*	*	*	*
KDGPEFTFTTPK	FAQGEIES	GASTKGRSV	EFLAPSSK	STS	GGTAALGCLV
670	680	690	700	710	720
*	*	*	*	*	*
SWNSGALTSGV	HTEPAVLOS	SGLYSLSSV	TVPSSSLG	TO	TYICNVNHK
730	740	750	760	770	780
*	*	*	*	*	*
PKSCDKTHTCP	PCPAPELLG	GPSVFLFP	PKPKDTLM	ISRT	PEVTCVVVDV
					SHEDPEVKFN

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Fig.9 (Cont).

790	800	810	820	830	840
*	*	*	*	*	*
<u>WYVDGVEVHNAKTKPREEOY NSTYRVVSVLTVLHODWLNG KEYKCKVSNKALPAPIEKTI</u>					
850	860	870	880	890	900
*	*	*	*	*	*
<u>SKAKGOPREPOVYTLPPSRD ELTKNOVSLTCLVKGFYPSD IAVEWESNGOPENNYKTTTP</u>					
910	920	930	940	950	
*	*	*	*	*	
<u>VLDSGDGSFFLYSKLTVDKSR WOOGNVFSCSVMEALHNHY TOKSLSLSPGK*</u>					

Fig.10.

Amino acid sequence of gp130Δ3fibro

Sequence Range: 1 to 332

10	20	30	40	50	60
*	*	*	*	*	*
MVTLQTWVQALFIFLTES TGELLDPCGYISPESPVVQL HSNFTAVCVLKEKCMDYFHV					
70	80	90	100	110	120
*	*	*	*	*	*
NANYIVWKTNHFTIPKEQYT IINRTASSVTFTDIASLNIQ LTCNILTFGQLEQNVIYGITI					
130	140	150	160	170	180
*	*	*	*	*	*
ISGLPPEKPKNLSCIVNEGK KMRCEWDGGRETHLETNFTL KSEWATHKFADCKAKRDTPT					
190	200	210	220	230	240
*	*	*	*	*	*
SCTVDYSTVYFVNIEVWVEA ENALGKVTSDHINFDPVYKV KPNPPHNLSVINSEELSSIL					
250	260	270	280	290	300
*	*	*	*	*	*
KLTWTNPSIKSVIILKYNIQ YRTKDASTWSQIPPEDTAST RSSFTVQDLKPFTEYVFRIR					
310	320	330			
*	*	*			
CMKEDGKGYWSDWSEEASGI TYEDRPSKAPSG					

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Fig.11.

Amino acid sequence of J-CH1

Sequence Range: 1 to 121

10	20	30	40	50	60
*	*	*	*	*	*
<u>SGGQGT</u> <u>LVTVSSASTKGPSV</u> <u>FPLAPSSKSTSGGTAALGCL</u> <u>VKDYFPEPVT</u> <u>TVSWNSGALTS</u>					
70	80	90	100	110	120
*	*	*	*	*	*
<u>GVHTFPAVLOSSGLYSLSSV</u> <u>VTVPSSSLGTOTYICNVNHK</u> <u>PSNTKVDKKVEPKSCDKTHT</u> *					

Fig.12.

Amino acid sequence of C γ 4

Sequence Range: 1 to 330

10	20	30	40	50	60
*	*	*	*	*	*
SGASTKGPSVFPLAPCSRST SESTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQ					
70	80	90	100	110	120
*	*	*	*	*	*
SSGLYSLSSVVTVPSSSLGT KTYTCNVDHKPSNTKVDKRV ESKYGPPCPSCPAPEFLGGP					
130	140	150	160	170	180
*	*	*	*	*	*
SVFLFPPKPKDTLMISRTPE VTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNS					
190	200	210	220	230	240
*	*	*	*	*	*
TYRVSVLTVLHQDWLNGKE YKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEM					
250	260	270	280	290	300
*	*	*	*	*	*
TKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVL DSDGSFFLYSRLTVDKSRWQ					
310	320	330			
*	*	*			
EGNVFSCSVMEALHNHYTQ KSLSLSLGK*					

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Fig.13.

Amino acid sequence of κ -domain

Sequence Range: 1 to 108

10	20	30	40	50	60
*	*	*	*	*	*
SGTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQ					
70	80	90	100		
*	*	*	*		
DSKDSTYSLSSTLTLSKADY EKHKVYACEVTHQGLSSPVT KSFNRGEC*					

Fig.14.

Amino acid sequence of λ -domain:

Sequence Range: 1 to 107

10	20	30	40	50	60
*	*	*	*	*	*
SGPKAAPSVTLFPPSSEELQ ANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSK					
70	80	90	100		
*	*	*	*		
QSNNKYAASSYLSLTPEQWK SHRSYSCQVTHEGSTVEKTV APTECS*					

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Fig.15.**Amino acid sequence of the soluble IL-6R α domain**

Sequence Range: 1 to 360

10	20	30	40	50	60
*	*	*	*	*	*
MVAVGCALLAALLAAPGAAL	APRRCPAQEVARGVLTSLPG	DSVTLTCPGVEPEDNATVHW			
70	80	90	100	110	120
*	*	*	*	*	*
VLRKPAAGSHPSRWAGMGRR	LLRSVQLHDSGNYSCYRAG	RPAGTVHLLVDVPPEEPQLS			
130	140	150	160	170	180
*	*	*	*	*	*
CFRKSPLSNVVCEWGPRSTP	SLTTKAVLLVRKFQNSPAED	FQEPQCYSQESQKFSCQLAV			
190	200	210	220	230	240
*	*	*	*	*	*
PEGDSSFYIVSMCVASSVGS	KFSKTQTFQGCILQPDPPA	NITVTAVARNPRWLSVTWQD			
250	260	270	280	290	300
*	*	*	*	*	*
PHSWNSSFYRLRFELRYRAE	RSKTFTTWMVKDLQHHCVIH	DAWSGLRHVVQLRAQEEFGQ			
310	320	330	340	350	360
*	*	*	*	*	*
GEWSEWSPEAMGTPWTESRS	PPAENEVSTPMQALTTNKDD	DNILFRDSANATSLPVQDAG			

Fig.16.**Amino acid sequence of the soluble IL-6k α 313 domain**

Sequence Range: 1 to 315

10	20	30	40	50	60
*	*	*	*	*	*
MVAVGCALLAALLAAPGAAL	APRRCPAQEVARGVLTSLPG	DSVTLTCPGVEPEDNATVHW			
70	80	90	100	110	120
*	*	*	*	*	*
VLRKPAAGSHPSRWAGMGRR	LLRSVQLHDSGNYSCYRAG	RPAGTVHLLVDVPPEEPQLS			
130	140	150	160	170	180
*	*	*	*	*	*
CFRKSPLSNVVCEWGPRSTP	SLTTKAVLLVRKFQNSPAED	FQEPQCYSQESQKFSCQLAV			
190	200	210	220	230	240
*	*	*	*	*	*
PEGDSSFYIVSMCVASSVGS	KFSKTQTFQGCILQPDPPA	NITVTAVARNPRWLSVTWQD			
250	260	270	280	290	300
*	*	*	*	*	*
PHSWNSSFYRLRFELRYRAE	RSKTFTTWMVKDLQHHCVIH	DAWSGLRHVVQLRAQEEFGQ			
310					
*					
GEWSEWSPEAMGTTG					

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Fig.17.

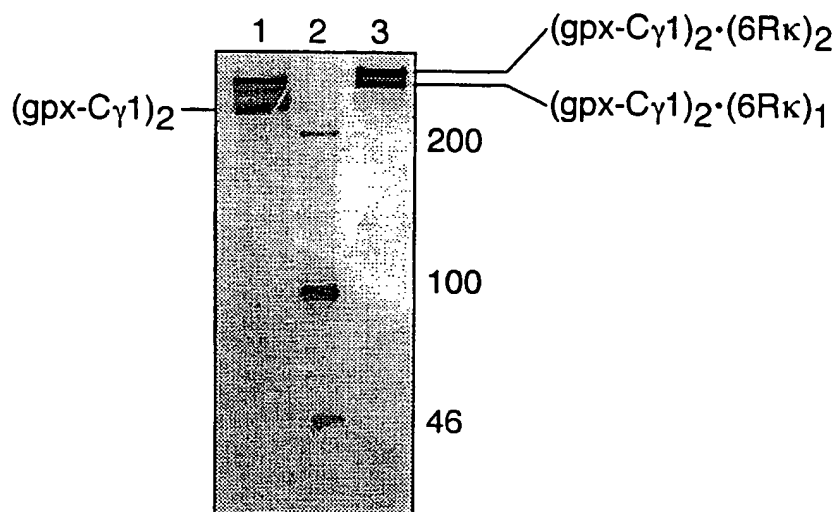


Fig.18.

IL-6 Dissociates Slowly from the Ligand Trap

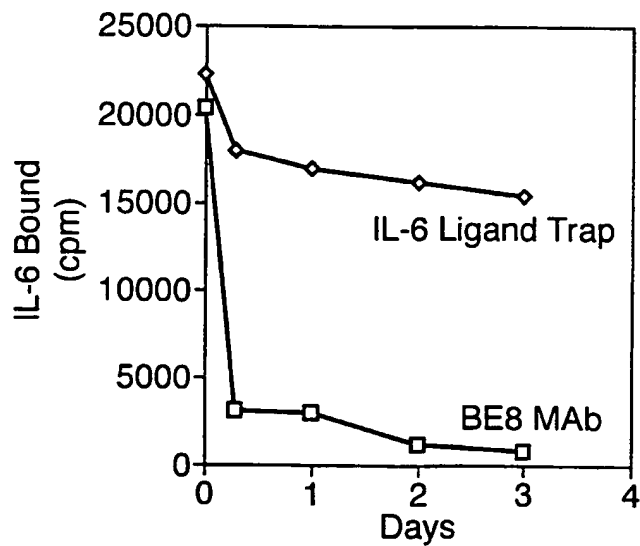


Fig. 19A.

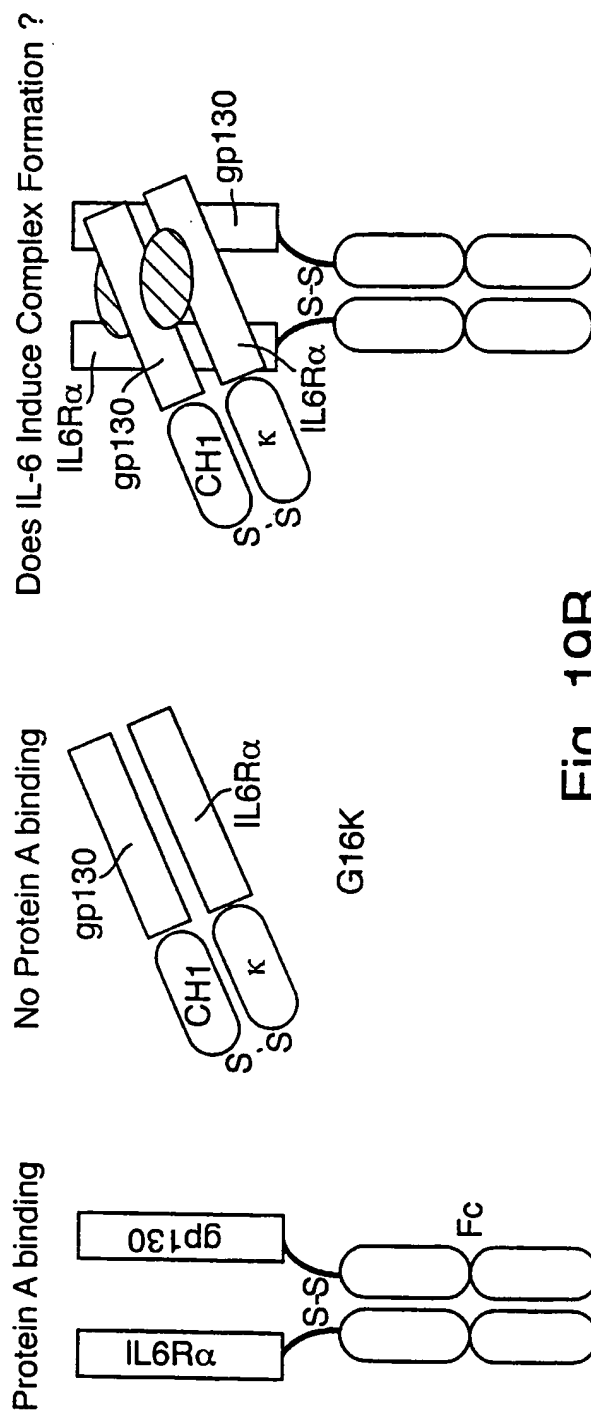
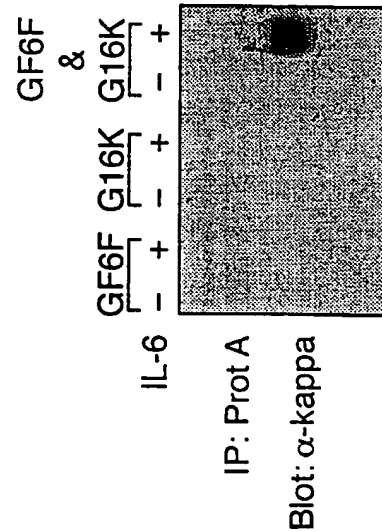


Fig. 19B.



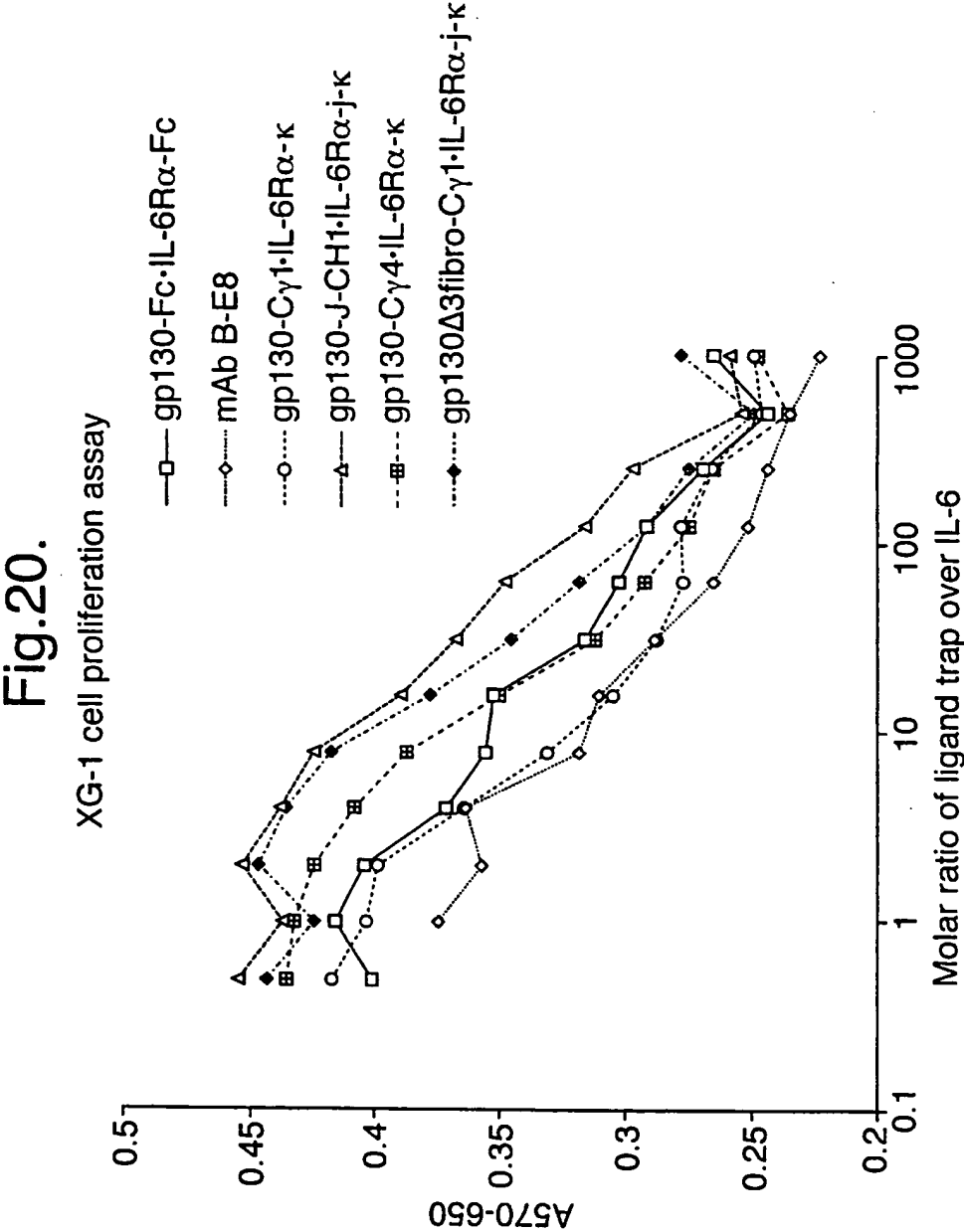


Fig.21A.

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      10      20      30      40
      *      *      *      *
ATG GTG AAG CCA TCA TTA CCA TTC ACA TCC CTC TTA TTC CTG CAG CTG
Met Val Lys Pro Ser Leu Pro Phe Thr Ser Leu Leu Phe Leu Gln Leu>

50      60      70      80      90
      *      *      *      *      *
CCC CTG CTG GGA GTG GGG CTG AAC ACG ACA ATT CTG ACG CCC AAT GGG
Pro Leu Leu Gly Val Gly Leu Asn Thr Thr Ile Leu Thr Pro Asn Gly>

100      110      120      130      140
      *      *      *      *      *
AAT GAA GAC ACC ACA GCT GAT TTC TTC CTG ACC ACT ATG CCC ACT GAC
Asn Glu Asp Thr Thr Ala Asp Phe Phe Leu Thr Thr Met Pro Thr Asp>

150      160      170      180      190
      *      *      *      *      *
TCC CTC AGT GTT TCC ACT CTG CCC CTC CCA GAG GTT CAG TGT TTT GTG
Ser Leu Ser Val Ser Thr Leu Pro Leu Pro Glu Val Gln Cys Phe Val>

200      210      220      230      240
      *      *      *      *      *
TTC AAT GTC GAG TAC ATG AAT TGC ACT TGG AAC AGC AGC TCT GAG CCC
Phe Asn Val Glu Tyr Met Asn Cys Thr Trp Asn Ser Ser Ser Glu Pro>

250      260      270      280
      *      *      *      *      *
CAG CCT ACC AAC CTC ACT CTG CAT TAT TGG TAC AAG AAC TCG GAT AAT
Gln Pro Thr Asn Leu Thr Leu His Tyr Trp Tyr Lys Asn Ser Asp Asn>

290      300      310      320      330
      *      *      *      *      *
GAT AAA GTC CAG AAG TGC AGC CAC TAT CTA TTC TCT GAA GAA ATC ACT
Asp Lys Val Gln Lys Cys Ser His Tyr Leu Phe Ser Glu Glu Ile Thr>

340      350      360      370      380
      *      *      *      *      *
TCT GGC TGT CAG TTG CAA AAA AAG GAG ATC CAC CTC TAC CAA ACA TTT
Ser Gly Cys Gln Leu Gln Lys Lys Glu Ile His Leu Tyr Gln Thr Phe>

390      400      410      420      430
      *      *      *      *      *
GTT GTT CAG CTC CAG GAC CCA CGG GAA CCC AGG AGA CAG GCC ACA CAG
Val Val Gln Leu Gln Asp Pro Arg Glu Pro Arg Arg Gln Ala Thr Gln>

440      450      460      470      480
      *      *      *      *      *
ATG CTA AAA CTG CAG AAT CTG GTG ATC CCC TGG GCT CCA GAG AAC CTA
Met Leu Lys Leu Gln Asn Leu Val Ile Pro Trp Ala Pro Glu Asn Leu>

490      500      510      520
      *      *      *      *      *
ACA CTT CAC AAA CTG AGT GAA TCC CAG CTA GAA CTG AAC TGG AAC AAC
Thr Leu His Lys Leu Ser Glu Ser Gln Leu Glu Leu Asn Trp Asn Asn>

530      540      550      560      570
      *      *      *      *      *
AGA TTC TTG AAC CAC TGT TTG GAG CAC TTG GTG CAG TAC CGG ACT GAC
Arg Phe Leu Asn His Cys Leu Glu His Leu Val Gln Tyr Arg Thr Asp>

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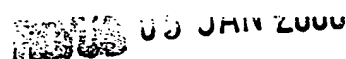


Fig.21B.

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580      590      600      610      620
*      *      *      *      *
TGG GAC CAC AGC TGG ACT GAA CAA TCA GTG GAT TAT AGA CAT AAG TTC
Trp Asp His Ser Trp Thr Glu Gln Ser Val Asp Tyr Arg His Lys Phe>

630      640      650      660      670
*      *      *      *      *
TCC TTG CCT AGT GTG GAT GGG CAG AAA CGC TAC ACG TTT CGT GTT CGG
Ser Leu Pro Ser Val Asp Gly Gln Lys Arg Tyr Thr Phe Arg Val Arg>

680      690      700      710      720
*      *      *      *      *
AGC CGC TTT AAC CCA CTC TGT GGA AGT GCT CAG CAT TGG AGT GAA TGG
Ser Arg Phe Asn Pro Leu Cys Gly Ser Ala Gln His Trp Ser Glu Trp>

730      740      750      760
*      *      *      *
AGC CAC CCA ATC CAC TGG GGG AGC AAT ACT TCA AAA GAG AAC GCG TCG
Ser His Pro Ile His Trp Gly Ser Asn Thr Ser Lys Glu Asn Ala Ser>

770      780      790      800      810
*      *      *      *      *
TCT GGG AAC ATG AAG GTC CTG CAG GAG CCC ACC TGC GTC TCC GAC TAC
Ser Gly Asn Met Lys Val Leu Gln Glu Pro Thr Cys Val Ser Asp Tyr>

820      830      840      850      860
*      *      *      *      *
ATG AGC ATC TCT ACT TGC GAG TGG AAG ATG AAT GGT CCC ACC AAT TGC
Met Ser Ile Ser Thr Cys Glu Trp Lys Met Asn Gly Pro Thr Asn Cys>

870      880      890      900      910
*      *      *      *      *
AGC ACC GAG CTC CGC CTG TTG TAC CAG CTG GTT TTT CTG CTC TCC GAA
Ser Thr Glu Leu Arg Leu Leu Tyr Gln Leu Val Phe Leu Leu Ser Glu>

920      930      940      950      960
*      *      *      *      *
GCC CAC ACG TGT ATC CCT GAG AAC AAC GGA GGC GCG GGG TGC GTG TGC
Ala His Thr Cys Ile Pro Glu Asn Asn Gly Gly Ala Gly Cys Val Cys>

970      980      990      1000
*      *      *      *
CAC CTG CTC ATG GAT GAC GTG GTC AGT GCG GAT AAC TAT ACA CTG GAC
His Leu Leu Met Asp Asp Val Val Ser Ala Asp Asn Tyr Thr Leu Asp>

1010      1020      1030      1040      1050
*      *      *      *      *
CTG TGG GCT GGG CAG CAG CTG CTG TGG AAG GGC TCC TTC AAG CCC AGC
Leu Trp Ala Gly Gln Gln Leu Leu Trp Lys Gly Ser Phe Lys Pro Ser>

1060      1070      1080      1090      1100
*      *      *      *      *
GAG CAT GTG AAA CCC AGG GCC CCA GGA AAC CTG ACA GTT CAC ACC AAT
Glu His Val Lys Pro Arg Ala Pro Gly Asn Leu Thr Val His Thr Asn>

1110      1120      1130      1140      1150
*      *      *      *      *
GTC TCC GAC ACT CTG CTG CTG ACC TGG AGC AAC CCG TAT CCC CCT GAC
Val Ser Asp Thr Leu Leu Leu Thr Trp Ser Asn Pro Tyr Pro Pro Asp>

1160      1170      1180      1190      1200
*      *      *      *      *

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Fig.21C.

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AAT TAC CTG TAT AAT CAT CTC ACC TAT GCA GTC AAC ATT TGG AGT GAA
Asn Tyr Leu Tyr Asn His Leu Thr Tyr Ala Val Asn Ile Trp Ser Glu>

      1210      1220      1230      1240
      *        *        *        *
AAC GAC CCG GCA GAT TTC AGA ATC TAT AAC GTG ACC TAC CTA GAA CCC
Asn Asp Pro Ala Asp Phe Arg Ile Tyr Asn Val Thr Tyr Leu Glu Pro>

1250      1260      1270      1280      1290
*        *        *        *        *
TCC CTC CGC ATC GCA GCC AGC ACC CTG AAG TCT GGG ATT TCC TAC AGG
Ser Leu Arg Ile Ala Ala Ser Thr Leu Lys Ser Gly Ile Ser Tyr Arg>

      1300      1310      1320      1330      1340
      *        *        *        *        *
GCA CGG GTG AGG GCC TGG GCT CAG TGC TAT AAC ACC ACC TGG AGT GAG
Ala Arg Val Arg Ala Trp Ala Gln Cys Tyr Asn Thr Thr Trp Ser Glu>

      1350      1360      1370      1380      1390
*        *        *        *        *
TGG AGC CCC AGC ACC AAG TGG CAC AAC TCC TAC AGG GAG CCC TTC GAG
Trp Ser Pro Ser Thr Lys Trp His Asn Ser Tyr Arg Glu Pro Phe Glu>

      1400      1410      1420      1430      1440
      *        *        *        *        *
CAG TCC GGA GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA
Gln Ser Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu>

      1450      1460      1470      1480
      *        *        *        *
CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp>

1490      1500      1510      1520      1530
*        *        *        *        *
ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp>

      1540      1550      1560      1570      1580
      *        *        *        *        *
GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly>

      1590      1600      1610      1620      1630
*        *        *        *        *
GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn>

      1640      1650      1660      1670      1680
      *        *        *        *        *
AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp>

      1690      1700      1710      1720
      *        *        *        *
CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro>

1730      1740      1750      1760      1770
*        *        *        *        *
GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu>

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Fig.21D.

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1780      1790      1800      1810      1820
*        *        *        *        *
CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAG GAG ATG ACC AAG AAC
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn>

1830      1840      1850      1860      1870
*        *        *        *        *
CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile>

1880      1890      1900      1910      1920
*        *        *        *        *
GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr>

1930      1940      1950      1960
*        *        *        *
ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAT AGC AAG
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys>

1970      1980      1990      2000      2010
*        *        *        *        *
CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys>

2020      2030      2040      2050      2060
*        *        *        *        *
TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu>

2070      2080
*        *        *        *
TCC CTG TCT CCG GGT AAA TGA
Ser Leu Ser Pro Gly Lys ***>

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Fig.22A.

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      10      20      30      40
      *      *      *      *
ATG GTG AAG CCA TCA TTA CCA TTC ACA TCC CTC TTA TTC CTG CAG CTG
Met Val Lys Pro Ser Leu Pro Phe Thr Ser Leu Leu Phe Leu Gln Leu>

50      60      70      80      90
      *      *      *      *      *
CCC CTG CTG GGA GTG GGG CTG AAC ACG ACA ATT CTG ACG CCC AAT GGG
Pro Leu Leu Gly Val Gly Leu Asn Thr Thr Ile Leu Thr Pro Asn Gly>

100     110     120     130     140
      *      *      *      *      *
AAT GAA GAC ACC ACA GCT GAT TTC TTC CTG ACC ACT ATG CCC ACT GAC
Asn Glu Asp Thr Thr Ala Asp Phe Phe Leu Thr Thr Met Pro Thr Asp>

150     160     170     180     190
      *      *      *      *      *
TCC CTC AGT GTT TCC ACT CTG CCC CTC CCA GAG GTT CAG TGT TTT GTG
Ser Leu Ser Val Ser Thr Leu Pro Leu Pro Glu Val Gln Cys Phe Val>

200     210     220     230     240
      *      *      *      *      *
TTC AAT GTC GAG TAC ATG AAT TGC ACT TGG AAC AGC AGC TCT GAG CCC
Phe Asn Val Glu Tyr Met Asn Cys Thr Trp Asn Ser Ser Ser Glu Pro>

250     260     270     280
      *      *      *      *      *
CAG CCT ACC AAC CTC ACT CTG CAT TAT TGG TAC AAG AAC TCG GAT AAT
Gln Pro Thr Asn Leu Thr Leu His Tyr Trp Tyr Lys Asn Ser Asp Asn>

290     300     310     320     330
      *      *      *      *      *
GAT AAA GTC CAG AAG TGC AGC CAC TAT CTA TTC TCT GAA GAA ATC ACT
Asp Lys Val Gln Lys Cys Ser His Tyr Leu Phe Ser Glu Glu Ile Thr>

340     350     360     370     380
      *      *      *      *      *
TCT GGC TGT CAG TTG CAA AAA AAG GAG ATC CAC CTC TAC CAA ACA TTT
Ser Gly Cys Gln Leu Gln Lys Lys Glu Ile His Leu Tyr Gln Thr Phe>

390     400     410     420     430
      *      *      *      *      *
GTT GTT CAG CTC CAG GAC CCA CGG GAA CCC AGG AGA CAG GCC ACA CAG
Val Val Gln Leu Gln Asp Pro Arg Glu Pro Arg Arg Gln Ala Thr Gln>

440     450     460     470     480
      *      *      *      *      *
ATG CTA AAA CTG CAG AAT CTG GTG ATC CCC TGG GCT CCA GAG AAC CTA
Met Leu Lys Leu Gln Asn Leu Val Ile Pro Trp Ala Pro Glu Asn Leu>

490     500     510     520
      *      *      *      *      *
ACA CTT CAC AAA CTG AGT GAA TCC CAG CTA GAA CTG AAC TGG AAC AAC
Thr Leu His Lys Leu Ser Glu Ser Gln Leu Glu Leu Asn Trp Asn Asn>

530     540     550     560     570
      *      *      *      *      *
AGA TTC TTG AAC CAC TGT TTG GAG CAC TTG GTG CAG TAC CGG ACT GAC
Arg Phe Leu Asn His Cys Leu Glu His Leu Val Gln Tyr Arg Thr Asp>

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Fig.22B.

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      580      590      600      610      620
      *      *      *      *      *
TGG GAC CAC AGC TGG ACT GAA CAA TCA GTG GAT TAT AGA CAT AAG TTC
Trp Asp His Ser Trp Thr Glu Gln Ser Val Asp Tyr Arg His Lys Phe>

      630      640      650      660      670
      *      *      *      *      *
TCC TTG CCT AGT GTG GAT GGG CAG AAA CGC TAC ACG TTT CGT GTT CGG
Ser Leu Pro Ser Val Asp Gly Gln Lys Arg Tyr Thr Phe Arg Val Arg>

      680      690      700      710      720
      *      *      *      *      *
AGC CGC TTT AAC CCA CTC TGT GGA AGT GCT CAG CAT TGG AGT GAA TGG
Ser Arg Phe Asn Pro Leu Cys Gly Ser Ala Gln His Trp Ser Glu Trp>

      730      740      750      760
      *      *      *      *      *
AGC CAC CCA ATC CAC TGG GGG AGC AAT ACT TCA AAA GAG AAC GGG AAC
Ser His Pro Ile His Trp Gly Ser Asn Thr Ser Lys Glu Asn Gly Asn>

770      780      790      800      810
*      *      *      *      *
ATG AAG GTC CTG CAG GAG CCC ACC TGC GTC TCC GAC TAC ATG AGC ATC
Met Lys Val Leu Gln Glu Pro Thr Cys Val Ser Asp Tyr Met Ser Ile>

      820      830      840      850      860
      *      *      *      *      *
TCT ACT TGC GAG TGG AAG ATG AAT GGT CCC ACC AAT TGC AGC ACC GAG
Ser Thr Cys Glu Trp Lys Met Asn Gly Pro Thr Asn Cys Ser Thr Glu>

      870      880      890      900      910
      *      *      *      *      *
CTC CGC CTG TTG TAC CAG CTG GTT TTT CTG CTC TCC GAA GCC CAC ACG
Leu Arg Leu Leu Tyr Gln Leu Val Phe Leu Leu Ser Glu Ala His Thr>

      920      930      940      950      960
      *      *      *      *      *
TGT ATC CCT GAG AAC AAC GGA GGC GCG GGG TGC GTG TGC CAC CTG CTC
Cys Ile Pro Glu Asn Asn Gly Gly Ala Gly Cys Val Cys His Leu Leu>

      970      980      990      1000
      *      *      *      *      *
ATG GAT GAC GTG GTC AGT GCG GAT AAC TAT ACA CTG GAC CTG TGG GCT
Met Asp Asp Val Val Ser Ala Asp Asn Tyr Thr Leu Asp Leu Trp Ala>

1010      1020      1030      1040      1050
*      *      *      *      *
GGG CAG CAG CTG CTG TGG AAG GGC TCC TTC AAG CCC AGC GAG CAT GTG
Gly Gln Gln Leu Leu Trp Lys Gly Ser Phe Lys Pro Ser Glu His Val>

      1060      1070      1080      1090      1100
      *      *      *      *      *
AAA CCC AGG GCC CCA GGA AAC CTG ACA GTT CAC ACC AAT GTC TCC GAC
Lys Pro Arg Ala Pro Gly Asn Leu Thr Val His Thr Asn Val Ser Asp>

      1110      1120      1130      1140      1150
      *      *      *      *      *
ACT CTG CTG CTG ACC TGG AGC AAC CCG TAT CCC CCT GAC AAT TAC CTG
Thr Leu Leu Leu Thr Trp Ser Asn Pro Tyr Pro Pro Asp Asn Tyr Leu>

      1160      1170      1180      1190      1200
      *      *      *      *      *

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Fig.22C.

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TAT AAT CAT CTC ACC TAT GCA GTC AAC ATT TGG AGT GAA AAC GAC CCG
Tyr Asn His Leu Thr Tyr Ala Val Asn Ile Trp Ser Glu Asn Asp Pro>

      1210      1220      1230      1240
      *      *      *      *      *      *
GCA GAT TTC AGA ATC TAT AAC GTG ACC TAC CTA GAA CCC TCC CTC CGC
Ala Asp Phe Arg Ile Tyr Asn Val Thr Tyr Leu Glu Pro Ser Leu Arg>

1250      1260      1270      1280      1290
*      *      *      *      *      *
ATC GCA GCC AGC ACC CTG AAG TCT GGG ATT TCC TAC AGG GCA CGG GTG
Ile Ala Ala Ser Thr Leu Lys Ser Gly Ile Ser Tyr Arg Ala Arg Val>

      1300      1310      1320      1330      1340
      *      *      *      *      *
AGG GCC TGG GCT CAG AGC TAT AAC ACC ACC TGG AGT GAG TGG AGC CCC
Arg Ala Trp Ala Gln Ser Tyr Asn Thr Thr Trp Ser Glu Trp Ser Pro>

      1350      1360      1370      1380      1390
      *      *      *      *      *
AGC ACC AAG TGG CAC AAC TCC TAC AGG GAG CCC TTC GAG CAG TCC GGA
Ser Thr Lys Trp His Asn Ser Tyr Arg Glu Pro Phe Glu Gln Ser Gly>

      1400      1410      1420      1430      1440
      *      *      *      *      *
GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly>

      1450      1460      1470      1480
      *      *      *      *      *
GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met>

1490      1500      1510      1520      1530
*      *      *      *      *
ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His>

      1540      1550      1560      1570      1580
      *      *      *      *      *
GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val>

      1590      1600      1610      1620      1630
      *      *      *      *      *
CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr>

      1640      1650      1660      1670      1680
      *      *      *      *      *
CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly>

      1690      1700      1710      1720
      *      *      *      *      *
AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile>

1730      1740      1750      1760      1770
*      *      *      *      *
GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val>

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Fig.22D.

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1780      1790      1800      1810      1820
  *          *          *          *          *
TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser>

1830      1840      1850      1860      1870
  *          *          *          *          *
CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu>

1880      1890      1900      1910      1920
  *          *          *          *          *
TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro>

1930      1940      1950      1960
  *          *          *          *          *
GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAT AGC AAG CTC ACC GTG
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val>

1970      1980      1990      2000      2010
  *          *          *          *          *
GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met>

2020      2030      2040      2050      2060
  *          *          *          *          *
CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser>

2070
  *          *          *
CCG GGT AAA TGA
Pro Gly Lys ***>

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Fig.23A.

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      10      20      30      40
      *      *      *      *
ATG GTG AAG CCA TCA TTA CCA TTC ACA TCC CTC TTA TTC CTG CAG CTG
Met Val Lys Pro Ser Leu Pro Phe Thr Ser Leu Leu Phe Leu Gln Leu>

50      60      70      80      90
      *      *      *      *      *
CCC CTG CTG GGA GTG GGG CTG AAC ACG ACA ATT CTG ACG CCC AAT GGG
Pro Leu Leu Gly Val Gly Leu Asn Thr Thr Ile Leu Thr Pro Asn Gly>

100     110     120     130     140
      *      *      *      *      *
AAT GAA GAC ACC ACA GCT GAT TTC TTC CTG ACC ACT ATG CCC ACT GAC
Asn Glu Asp Thr Thr Ala Asp Phe Phe Leu Thr Thr Met Pro Thr Asp>

150     160     170     180     190
      *      *      *      *      *
TCC CTC AGT GTT TCC ACT CTG CCC CTC CCA GAG GTT CAG TGT TTT GTG
Ser Leu Ser Val Ser Thr Leu Pro Leu Pro Glu Val Gln Cys Phe Val>

200     210     220     230     240
      *      *      *      *      *
TTC AAT GTC GAG TAC ATG AAT TGC ACT TGG AAC AGC AGC TCT GAG CCC
Phe Asn Val Glu Tyr Met Asn Cys Thr Trp Asn Ser Ser Ser Glu Pro>

250     260     270     280
      *      *      *      *
CAG CCT ACC AAC CTC ACT CTG CAT TAT TGG TAC AAG AAC TCG GAT AAT
Gln Pro Thr Asn Leu Thr Leu His Tyr Trp Tyr Lys Asn Ser Asp Asn>

290     300     310     320     330
      *      *      *      *      *
GAT AAA GTC CAG AAG TGC AGC CAC TAT CTA TTC TCT GAA GAA ATC ACT
Asp Lys Val Gln Lys Cys Ser His Tyr Leu Phe Ser Glu Glu Ile Thr>

340     350     360     370     380
      *      *      *      *      *
TCT GGC TGT CAG TTG CAA AAA AAG GAG ATC CAC CTC TAC CAA ACA TTT
Ser Gly Cys Gln Leu Gln Lys Lys Glu Ile His Leu Tyr Gln Thr Phe>

390     400     410     420     430
      *      *      *      *      *
GTT GTT CAG CTC CAG GAC CCA CGG GAA CCC AGG AGA CAG GCC ACA CAG
Val Val Gln Leu Gln Asp Pro Arg Glu Pro Arg Arg Gln Ala Thr Gln>

440     450     460     470     480
      *      *      *      *      *
ATG CTA AAA CTG CAG AAT CTG GTG ATC CCC TGG GCT CCA GAG AAC CTA
Met Leu Lys Leu Gln Asn Leu Val Ile Pro Trp Ala Pro Glu Asn Leu>

490     500     510     520
      *      *      *      *
ACA CTT CAC AAA CTG AGT GAA TCC CAG CTA GAA CTG AAC TGG AAC AAC
Thr Leu His Lys Leu Ser Glu Ser Gln Leu Glu Leu Asn Trp Asn Asn>

530     540     550     560     570
      *      *      *      *      *
AGA TTC TTG AAC CAC TGT TTG GAG CAC TTG GTG CAG TAC CGG ACT GAC
Arg Phe Leu Asn His Cys Leu Glu His Leu Val Gln Tyr Arg Thr Asp>

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Fig.23B.

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580      590      600      610      620
*        *        *        *        *
TGG GAC CAC AGC TGG ACT GAA CAA TCA GTG GAT TAT AGA CAT AAG TTC
Trp Asp His Ser Trp Thr Glu Gln Ser Val Asp Tyr Arg His Lys Phe>

630      640      650      660      670
*        *        *        *        *
TCC TTG CCT AGT GTG GAT GGG CAG AAA CGC TAC ACG TTT CGT GTT CGG
Ser Leu Pro Ser Val Asp Gly Gln Lys Arg Tyr Thr Phe Arg Val Arg>

680      690      700      710      720
*        *        *        *        *
AGC CGC TTT AAC CCA CTC TGT GGA AGT GCT CAG CAT TGG AGT GAA TGG
Ser Arg Phe Asn Pro Leu Cys Gly Ser Ala Gln His Trp Ser Glu Trp>

730      740      750      760
*        *        *        *
AGC CAC CCA ATC CAC TGG GGG AGC AAT ACT TCA AAA GAG AAC GCG TCG
Ser His Pro Ile His Trp Gly Ser Asn Thr Ser Lys Glu Asn Ala Ser>

770      780      790      800      810
*        *        *        *        *
TCT GGG AAC ATG AAG GTC CTG CAG GAG CCC ACC TGC GTC TCC GAC TAC
Ser Gly Asn Met Lys Val Leu Gln Glu Pro Thr Cys Val Ser Asp Tyr>

820      830      840      850      860
*        *        *        *        *
ATG AGC ATC TCT ACT TGC GAG TGG AAG ATG AAT GGT CCC ACC AAT TGC
Met Ser Ile Ser Thr Cys Glu Trp Lys Met Asn Gly Pro Thr Asn Cys>

870      880      890      900      910
*        *        *        *        *
AGC ACC GAG CTC CGC CTG TTG TAC CAG CTG GTT TTT CTG CTC TCC GAA
Ser Thr Glu Leu Arg Leu Leu Tyr Gln Leu Val Phe Leu Leu Ser Glu>

920      930      940      950      960
*        *        *        *        *
GCC CAC ACG TGT ATC CCT GAG AAC AAC GGA GGC GCG GGG TGC GTG TGC
Ala His Thr Cys Ile Pro Glu Asn Asn Gly Gly Ala Gly Cys Val Cys>

970      980      990      1000
*        *        *        *
CAC CTG CTC ATG GAT GAC GTG GTC AGT GCG GAT AAC TAT ACA CTG GAC
His Leu Leu Met Asp Asp Val Val Ser Ala Asp Asn Tyr Thr Leu Asp>

1010      1020      1030      1040      1050
*        *        *        *        *
CTG TGG GCT GGG CAG CAG CTG CTG TGG AAG GGC TCC TTC AAG CCC AGC
Leu Trp Ala Gly Gln Gln Leu Leu Trp Lys Gly Ser Phe Lys Pro Ser>

1060      1070      1080      1090      1100
*        *        *        *        *
GAG CAT GTG AAA CCC AGG GCC CCA GGA AAC CTG ACA GTT CAC ACC AAT
Glu His Val Lys Pro Arg Ala Pro Gly Asn Leu Thr Val His Thr Asn>

1110      1120      1130      1140      1150
*        *        *        *        *
GTC TCC GAC ACT CTG CTG CTG ACC TGG AGC AAC CCG TAT CCC CCT GAC
Val Ser Asp Thr Leu Leu Leu Thr Trp Ser Asn Pro Tyr Pro Pro Asp>

1160      1170      1180      1190      1200
*        *        *        *        *

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Fig.23C.

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AAT TAC CTG TAT AAT CAT CTC ACC TAT GCA GTC AAC ATT TGG AGT GAA
Asn Tyr Leu Tyr Asn His Leu Thr Tyr Ala Val Asn Ile Trp Ser Glu>

      1210      1220      1230      1240
      *      *      *      *
AAC GAC CCG GCA GAT TTC AGA ATC TAT AAC GTG ACC TAC CTA GAA CCC
Asn Asp Pro Ala Asp Phe Arg Ile Tyr Asn Val Thr Tyr Leu Glu Pro>

1250      1260      1270      1280      1290
*      *      *      *      *
TCC CTC CGC ATC GCA GCC AGC ACC CTG AAG TCT GGG ATT TCC TAC AGG
Ser Leu Arg Ile Ala Ala Ser Thr Leu Lys Ser Gly Ile Ser Tyr Arg>

      1300      1310      1320      1330      1340
      *      *      *      *      *
GCA CGG GTG AGG GCC TGG GCT CAG AGC TAT AAC ACC ACC TGG AGT GAG
Ala Arg Val Arg Ala Trp Ala Gln Ser Tyr Asn Thr Thr Trp Ser Glu>

      1350      1360      1370      1380      1390
      *      *      *      *      *
TGG AGC CCC AGC ACC AAG TGG CAC AAC TCC TAC AGG GAG CCC TTC GAG
Trp Ser Pro Ser Thr Lys Trp His Asn Ser Tyr Arg Glu Pro Phe Glu>

      1400      1410      1420      1430      1440
      *      *      *      *      *
CAG TCC GGA GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA
Gln Ser Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu>

      1450      1460      1470      1480
      *      *      *      *
CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp>

1490      1500      1510      1520      1530
*      *      *      *      *
ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp>

      1540      1550      1560      1570      1580
      *      *      *      *      *
GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly>

      1590      1600      1610      1620      1630
      *      *      *      *      *
GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn>

      1640      1650      1660      1670      1680
      *      *      *      *      *
AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp>

      1690      1700      1710      1720
      *      *      *      *
CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro>

1730      1740      1750      1760      1770
*      *      *      *      *
GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu>

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Fig.23D.

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1780      1790      1800      1810      1820
*          *          *          *          *
CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn>

1830      1840      1850      1860      1870
*          *          *          *          *
CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile>

1880      1890      1900      1910      1920
*          *          *          *          *
GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr>

1930      1940      1950      1960
*          *          *          *          *
ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAT AGC AAG
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys>

1970      1980      1990      2000      2010
*          *          *          *          *
CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys>

2020      2030      2040      2050      2060
*          *          *          *          *
TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu>

2070      2080
*          *          *          *          *
TCC CTG TCT CCG GGT AAA TGA
Ser Leu Ser Pro Gly Lys ***>

```

Fig.24A.

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      10      20      30      40
      *      *      *      *
ATG GTG GCC GTC GGC TGC GCG CTG CTG GCT GCC CTG CTG GCC GCG CCG
Met Val Ala Val Gly Cys Ala Leu Leu Ala Ala Leu Leu Ala Ala Pro>

50      60      70      80      90
      *      *      *      *      *
GGA GCG GCG CTG GCC CCA AGG CGC TGC CCT GCG CAG GAG GTG GCA AGA
Gly Ala Ala Leu Ala Pro Arg Arg Cys Pro Ala Gln Glu Val Ala Arg>

100     110     120     130     140
      *      *      *      *      *
GGC GTG CTG ACC AGT CTG CCA GGA GAC AGC GTG ACT CTG ACC TGC CCG
Gly Val Leu Thr Ser Leu Pro Gly Asp Ser Val Thr Leu Thr Cys Pro>

150     160     170     180     190
      *      *      *      *      *
GGG GTA GAG CCG GAA GAC AAT GCC ACT GTT CAC TGG GTG CTC AGG AAG
Gly Val Glu Pro Glu Asp Asn Ala Thr Val His Trp Val Leu Arg Lys>

200     210     220     230     240
      *      *      *      *      *
CCG GCT GCA GGC TCC CAC CCC AGC AGA TGG GCT GGC ATG GGA AGG AGG
Pro Ala Ala Gly Ser His Pro Ser Arg Trp Ala Gly Met Gly Arg Arg>

250     260     270     280
      *      *      *      *      *
CTG CTG CTG AGG TCG GTG CAG CTC CAC GAC TCT GGA AAC TAT TCA TGC
Leu Leu Leu Arg Ser Val Gln Leu His Asp Ser Gly Asn Tyr Ser Cys>

290     300     310     320     330
      *      *      *      *      *
TAC CGG GCC GGC CGC CCA GCT GGG ACT GTG CAC TTG CTG GTG GAT GTT
Tyr Arg Ala Gly Arg Pro Ala Gly Thr Val His Leu Leu Val Asp Val>

340     350     360     370     380
      *      *      *      *      *
CCC CCC GAG GAG CCC CAG CTC TCC TGC TTC CGG AAG AGC CCC CTC AGC
Pro Pro Glu Glu Pro Gln Leu Ser Cys Phe Arg Lys Ser Pro Leu Ser>

390     400     410     420     430
      *      *      *      *      *
AAT GTT GTT TGT GAG TGG GGT CCT CGG AGC ACC CCA TCC CTG ACG ACA
Asn Val Val Cys Glu Trp Gly Pro Arg Ser Thr Pro Ser Leu Thr Thr>

440     450     460     470     480
      *      *      *      *      *
AAG GCT GTG CTC TTG GTG AGG AAG TTT CAG AAC AGT CCG GCC GAA GAC
Lys Ala Val Leu Leu Val Arg Lys Phe Gln Asn Ser Pro Ala Glu Asp>

490     500     510     520
      *      *      *      *      *
TTC CAG GAG CCG TGC CAG TAT TCC CAG GAG TTC CAG AAG TTC TCC TGC
Phe Gln Glu Pro Cys Gln Tyr Ser Gln Glu Ser Gln Lys Phe Ser Cys>

530     540     550     560     570
      *      *      *      *      *
CAG TTA GCA GTC CCG GAG GGA GAC AGC TCT TTC TAC ATA GTG TCC ATG
Gln Leu Ala Val Pro Glu Gly Asp Ser Ser Phe Tyr Ile Val Ser Met>

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Fig.24B.

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580      590      600      610      620
*      *      *      *      *
TGC GTC GCC AGT AGT GTC GGG AGC AAG TTC AGC AAA ACT CAA ACC TTT
Cys Val Ala Ser Ser Val Gly Ser Lys Phe Ser Lys Thr Gln Thr Phe>

630      640      650      660      670
*      *      *      *      *
CAG GGT TGT GGA ATC TTG CAG CCT GAT CCG CCT GCC AAC ATC ACA GTC
Gln Gly Cys Gly Ile Leu Gln Pro Asp Pro Pro Ala Asn Ile Thr Val>

680      690      700      710      720
*      *      *      *      *
ACT GCC GTG GCC AGA AAC CCC CGC TGG CTC AGT GTC ACC TGG CAA GAC
Thr Ala Val Ala Arg Asn Pro Arg Trp Leu Ser Val Thr Trp Gln Asp>

730      740      750      760
*      *      *      *      *
CCC CAC TCC TGG AAC TCA TCT TTC TAC AGA CTA CGG TTT GAG CTC AGA
Pro His Ser Trp Asn Ser Ser Phe Tyr Arg Leu Arg Phe Glu Leu Arg>

770      780      790      800      810
*      *      *      *      *
TAT CGG GCT GAA CGG TCA AAG ACA TTC ACA ACA TGG ATG GTC AAG GAC
Tyr Arg Ala Glu Arg Ser Lys Thr Phe Thr Thr Trp Met Val Lys Asp>

820      830      840      850      860
*      *      *      *      *
CTC CAG CAT CAC TGT GTC ATC CAC GAC GCC TGG AGC GGC CTG AGG CAC
Leu Gln His His Cys Val Ile His Asp Ala Trp Ser Gly Leu Arg His>

870      880      890      900      910
*      *      *      *      *
GTG GTG CAG CTT CGT GCC CAG GAG GAG TTC GGG CAA GGC GAG TGG AGC
Val Val Gln Leu Arg Ala Gln Glu Glu Phe Gly Gln Gly Glu Trp Ser>

920      930      940      950      960
*      *      *      *      *
GAG TGG AGC CCG GAG GCC ATG GGC ACG CCT TGG ACA GAA TCC AGG AGT
Glu Trp Ser Pro Glu Ala Met Gly Thr Pro Trp Thr Glu Ser Arg Ser>

970      980      990      1000
*      *      *      *      *
CCT CCA GCT GAG AAC GAG GTG TCC ACC CCC ATG ACC GGT GGC GCG CCT
Pro Pro Ala Glu Asn Glu Val Ser Thr Pro Met Thr Gly Gly Ala Pro>

1010      1020      1030      1040      1050
*      *      *      *      *
TCA GGT GCT CAG CTG GAA CTT CTA GAC CCA TGT GGT TAT ATC AGT CCT
Ser Gly Ala Gln Leu Glu Leu Leu Asp Pro Cys Gly Tyr Ile Ser Pro>

1060      1070      1080      1090      1100
*      *      *      *      *
GAA TCT CCA GTT GTA CAA CTT CAT TCT AAT TTC ACT GCA GTT TGT GTG
Glu Ser Pro Val Val Gln Leu His Ser Asn Phe Thr Ala Val Cys Val>

1110      1120      1130      1140      1150
*      *      *      *      *
CTA AAG GAA AAA TGT ATG GAT TAT TTT CAT GTA AAT GCT AAT TAC ATT
Leu Lys Glu Lys Cys Met Asp Tyr Phe His Val Asn Ala Asn Tyr Ile>

1160      1170      1180      1190      1200
*      *      *      *      *

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Fig.24C.

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GTC TGG AAA ACA AAC CAT TTT ACT ATT CCT AAG GAG CAA TAT ACT ATC
 Val Trp Lys Thr Asn His Phe Thr Ile Pro Lys Glu Gln Tyr Thr Ile>

1210 1220 1230 1240
 * * * * *
 ATA AAC AGA ACA GCA TCC AGT GTC ACC TTT ACA GAT ATA GCT TCA TTA
 Ile Asn Arg Thr Ala Ser Ser Val Thr Phe Thr Asp Ile Ala Ser Leu>

1250 1260 1270 1280 1290
 * * * * *
 AAT ATT CAG CTC ACT TGC AAC ATT CTT ACA TTC GGA CAG CTT GAA CAG
 Asn Ile Gln Leu Thr Cys Asn Ile Leu Thr Phe Gly Gln Leu Glu Gln>

1300 1310 1320 1330 1340
 * * * * *
 AAT GTT TAT GGA ATC ACA ATA ATT TCA GGC TTG CCT CCA GAA AAA CCT
 Asn Val Tyr Gly Ile Thr Ile Ile Ser Gly Leu Pro Pro Glu Lys Pro>

1350 1360 1370 1380 1390
 * * * * *
 AAA AAT TTG AGT TGC ATT GTG AAC GAG GGG AAG AAA ATG AGG TGT GAG
 Lys Asn Leu Ser Cys Ile Val Asn Glu Gly Lys Lys Met Arg Cys Glu>

1400 1410 1420 1430 1440
 * * * * *
 TGG GAT GGT GGA AGG GAA ACA CAC TTG GAG ACA AAC TTC ACT TTA AAA
 Trp Asp Gly Gly Arg Glu Thr His Leu Glu Thr Asn Phe Thr Leu Lys>

1450 1460 1470 1480
 * * * * *
 TCT GAA TGG GCA ACA CAC AAG TTT GCT GAT TGC AAA GCA AAA CGT GAC
 Ser Glu Trp Ala Thr His Lys Phe Ala Asp Cys Lys Ala Lys Arg Asp>

1490 1500 1510 1520 1530
 * * * * *
 ACC CCC ACC TCA TGC ACT GTT GAT TAT TCT ACT GTG TAT TTT GTC AAC
 Thr Pro Thr Ser Cys Thr Val Asp Tyr Ser Thr Val Tyr Phe Val Asn>

1540 1550 1560 1570 1580
 * * * * *
 ATT GAA GTC TGG GTA GAA GCA GAG AAT GCC CTT GGG AAG GTT ACA TCA
 Ile Glu Val Trp Val Glu Ala Glu Asn Ala Leu Gly Lys Val Thr Ser>

1590 1600 1610 1620 1630
 * * * * *
 GAT CAT ATC AAT TTT GAT CCT GTA TAT AAA GTG AAG CCC AAT CCG CCA
 Asp His Ile Asn Phe Asp Pro Val Tyr Lys Val Lys Pro Asn Pro Pro>

1640 1650 1660 1670 1680
 * * * * *
 CAT AAT TTA TCA GTG ATC AAC TCA GAG GAA CTG TCT AGT ATC TTA AAA
 His Asn Leu Ser Val Ile Asn Ser Glu Glu Leu Ser Ser Ile Leu Lys>

1690 1700 1710 1720
 * * * * *
 TTG ACA TGG ACC AAC CCA AGT ATT AAG AGT GTT ATA ATA CTA AAA TAT
 Leu Thr Trp Thr Asn Pro Ser Ile Lys Ser Val Ile Ile Leu Lys Tyr>

1730 1740 1750 1760 1770
 * * * * *
 AAC ATT CAA TAT AGG ACC AAA GAT GCC TCA ACT TGG AGC CAG ATT CCT
 Asn Ile Gln Tyr Arg Thr Lys Asp Ala Ser Thr Trp Ser Gln Ile Pro>

Fig.24D.

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1780      1790      1800      1810      1820
*          *          *          *          *
CCT GAA GAC ACA GCA TCC ACC CGA TCT TCA TTC ACT GTC CAA GAC CTT
Pro Glu Asp Thr Ala Ser Thr Arg Ser Ser Phe Thr Val Gln Asp Leu>

1830      1840      1850      1860      1870
*          *          *          *          *
AAA CCT TTT ACA GAA TAT GTG TTT AGG ATT CGC TGT ATG AAG GAA GAT
Lys Pro Phe Thr Glu Tyr Val Phe Arg Ile Arg Cys Met Lys Glu Asp>

1880      1890      1900      1910      1920
*          *          *          *          *
GGT AAG GGA TAC TGG AGT GAC TGG AGT GAA GAA GCA AGT GGG ATC ACC
Gly Lys Gly Tyr Trp Ser Asp Trp Ser Glu Glu Ala Ser Gly Ile Thr>

1930      1940      1950      1960
*          *          *          *          *
TAT GAA GAT AGA CCA TCT AAA GCA CCA AGT TTC TGG TAT AAA ATA GAT
Tyr Glu Asp Arg Pro Ser Lys Ala Pro Ser Phe Trp Tyr Lys Ile Asp>

1970      1980      1990      2000      2010
*          *          *          *          *
CCA TCC CAT ACT CAA GGC TAC AGA ACT GTA CAA CTC GTG TGG AAG ACA
Pro Ser His Thr Gln Gly Tyr Arg Thr Val Gln Leu Val Trp Lys Thr>

2020      2030      2040      2050      2060
*          *          *          *          *
TTG CCT CCT TTT GAA GCC AAT GGA AAA ATC TTG GAT TAT GAA GTG ACT
Leu Pro Pro Phe Glu Ala Asn Gly Lys Ile Leu Asp Tyr Glu Val Thr>

2070      2080      2090      2100      2110
*          *          *          *          *
CTC ACA AGA TGG AAA TCA CAT TTA CAA AAT TAC ACA GTT AAT GCC ACA
Leu Thr Arg Trp Lys Ser His Leu Gln Asn Tyr Thr Val Asn Ala Thr>

2120      2130      2140      2150      2160
*          *          *          *          *
AAA CTG ACA GTA AAT CTC ACA AAT GAT CGC TAT CTA GCA ACC CTA ACA
Lys Leu Thr Val Asn Leu Thr Asn Asp Arg Tyr Leu Ala Thr Leu Thr>

2170      2180      2190      2200
*          *          *          *          *
GTA AGA AAT CTT GTT GGC AAA TCA GAT GCA GCT GTT TTA ACT ATC CCT
Val Arg Asn Leu Val Gly Lys Ser Asp Ala Ala Val Leu Thr Ile Pro>

2210      2220      2230      2240      2250
*          *          *          *          *
GCC TGT GAC TTT CAA GCT ACT CAC CCT GTA ATG GAT CTT AAA GCA TTC
Ala Cys Asp Phe Gln Ala Thr His Pro Val Met Asp Leu Lys Ala Phe>

2260      2270      2280      2290      2300
*          *          *          *          *
CCC AAA GAT AAC ATG CTT TGG GTG GAA TGG ACT ACT CCA AGG GAA TCT
Pro Lys Asp Asn Met Leu Trp Val Glu Trp Thr Thr Pro Arg Glu Ser>

2310      2320      2330      2340      2350
*          *          *          *          *
GTA AAG AAA TAT ATA CTT GAG TGG TGT GTG TTA TCA GAT AAA GCA CCC
Val Lys Lys Tyr Ile Leu Glu Trp Cys Val Leu Ser Asp Lys Ala Pro>

2360      2370      2380      2390      2400

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Fig.24E.

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*      *      *      *      *      *      *      *      *      *
TGT ATC ACA GAC TGG CAA CAA GAA GAT GGT ACC GTG CAT CGC ACC TAT
Cys Ile Thr Asp Trp Gln Gln Glu Asp Gly Thr Val His Arg Thr Tyr>

      2410      2420      2430      2440
*      *      *      *      *      *      *      *
TTA AGA GGG AAC TTA GCA GAG AGC AAA TGC TAT TTG ATA ACA GTT ACT
Leu Arg Gly Asn Leu Ala Glu Ser Lys Cys Tyr Leu Ile Thr Val Thr>

2450      2460      2470      2480      2490
*      *      *      *      *      *      *      *
CCA GTA TAT GCT GAT GGA CCA GGA AGC CCT GAA TCC ATA AAG GCA TAC
Pro Val Tyr Ala Asp Gly Pro Gly Ser Pro Glu Ser Ile Lys Ala Tyr>

      2500      2510      2520      2530      2540
*      *      *      *      *      *      *      *
CTT AAA CAA GCT CCA CCT TCC AAA GGA CCT ACT GTT CGG ACA AAA AAA
Leu Lys Gln Ala Pro Pro Ser Lys Gly Pro Thr Val Arg Thr Lys Lys>

      2550      2560      2570      2580      2590
*      *      *      *      *      *      *      *
GTA GGG AAA AAC GAA GCT GTC TTA GAG TGG GAC CAA CTT CCT GTT GAT
Val Gly Lys Asn Glu Ala Val Leu Glu Trp Asp Gln Leu Pro Val Asp>

      2600      2610      2620      2630      2640
*      *      *      *      *      *      *      *
GTT CAG AAT GGA TTT ATC AGA AAT TAT ACT ATA TTT TAT AGA ACC ATC
Val Gln Asn Gly Phe Ile Arg Asn Tyr Thr Ile Phe Tyr Arg Thr Ile>

      2650      2660      2670      2680
*      *      *      *      *      *      *      *
ATT GGA AAT GAA ACT GCT GTG AAT GTG GAT TCT TCC CAC ACA GAA TAT
Ile Gly Asn Glu Thr Ala Val Asn Val Asp Ser Ser His Thr Glu Tyr>

2690      2700      2710      2720      2730
*      *      *      *      *      *      *      *
ACA TTG TCC TCT TTG ACT AGT GAC ACA TTG TAC ATG GTA CGA ATG GCA
Thr Leu Ser Ser Leu Thr Ser Asp Thr Leu Tyr Met Val Arg Met Ala>

      2740      2750      2760      2770      2780
*      *      *      *      *      *      *      *
GCA TAC ACA GAT GAA GGT GGG AAG GAT GGT CCA GAA TTC ACT TTT ACT
Ala Tyr Thr Asp Glu Gly Gly Lys Asp Gly Pro Glu Phe Thr Phe Thr>

      2790      2800      2810      2820      2830
*      *      *      *      *      *      *      *
ACC CCA AAG TTT GCT CAA GGA GAA ATT GAA TCC GGG GGC GAC AAA ACT
Thr Pro Lys Phe Ala Gln Gly Glu Ile Glu Ser Gly Gly Asp Lys Thr>

      2840      2850      2860      2870      2880
*      *      *      *      *      *      *      *
CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser>

      2890      2900      2910      2920
*      *      *      *      *      *      *      *
GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg>

2930      2940      2950      2960      2970
*      *      *      *      *      *      *      *
ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT

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Fig.24F.

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Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro>
2980      2990      3000      3010      3020
*          *          *          *          *
GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala>
3030      3040      3050      3060      3070
*          *          *          *          *
AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val>
3080      3090      3100      3110      3120
*          *          *          *          *
AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr>
3130      3140      3150      3160
*          *          *          *          *
AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr>
3170      3180      3190      3200      3210
*          *          *          *          *
ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu>
3220      3230      3240      3250      3260
*          *          *          *          *
CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC
Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys>
3270      3280      3290      3300      3310
*          *          *          *          *
CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser>
3320      3330      3340      3350      3360
*          *          *          *          *
AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp>
3370      3380      3390      3400
*          *          *          *          *
TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser>
3410      3420      3430      3440      3450
*          *          *          *          *
AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala>
3460      3470      3480      3490      3500
*          *          *          *          *
CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys>
*
TGA
***>

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Fig.25A.

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      10      20      30      40
      *      *      *      *
ATG GTG GCC GTC GGC TGC GCG CTG CTG GCT GCC CTG CTG GCC GCG CCG
Met Val Ala Val Gly Cys Ala Leu Leu Ala Ala Leu Leu Ala Ala Pro>

50      60      70      80      90
      *      *      *      *      *
GGA GCG GCG CTG GCC CCA AGG CGC TGC CCT GCG CAG GAG GTG GCA AGA
Gly Ala Ala Leu Ala Pro Arg Arg Cys Pro Ala Gln Glu Val Ala Arg>

100     110     120     130     140
      *      *      *      *      *
GGC GTG CTG ACC AGT CTG CCA GGA GAC AGC GTG ACT CTG ACC TGC CCG
Gly Val Leu Thr Ser Leu Pro Gly Asp Ser Val Thr Leu Thr Cys Pro>

150     160     170     180     190
      *      *      *      *      *
GGG GTA GAG CCG GAA GAC AAT GCC ACT GTT CAC TGG GTG CTC AGG AAG
Gly Val Glu Pro Glu Asp Asn Ala Thr Val His Trp Val Leu Arg Lys>

200     210     220     230     240
      *      *      *      *      *
CCG GCT GCA GGC TCC CAC CCC AGC AGA TGG GCT GGC ATG GGA AGG AGG
Pro Ala Ala Gly Ser His Pro Ser Arg Trp Ala Gly Met Gly Arg Arg>

250     260     270     280
      *      *      *      *      *
CTG CTG CTG AGG TCG GTG CAG CTC CAC GAC TCT GGA AAC TAT TCA TGC
Leu Leu Leu Arg Ser Val Gln Leu His Asp Ser Gly Asn Tyr Ser Cys>

290     300     310     320     330
      *      *      *      *      *
TAC CGG GCC GGC CGC CCA GCT GGG ACT GTG CAC TTG CTG GTG GAT GTT
Tyr Arg Ala Gly Arg Pro Ala Gly Thr Val His Leu Leu Val Asp Val>

340     350     360     370     380
      *      *      *      *      *
CCC CCC GAG GAG CCC CAG CTC TCC TGC TTC CGG AAG AGC CCC CTC AGC
Pro Pro Glu Glu Pro Gln Leu Ser Cys Phe Arg Lys Ser Pro Leu Ser>

390     400     410     420     430
      *      *      *      *      *
AAT GTT GTT TGT GAG TGG GGT CCT CGG AGC ACC CCA TCC CTG ACG ACA
Asn Val Val Cys Glu Trp Gly Pro Arg Ser Thr Pro Ser Leu Thr Thr>

440     450     460     470     480
      *      *      *      *      *
AAG GCT GTG CTC TTG GTG AGG AAG TTT CAG AAC AGT CCG GCC GAA GAC
Lys Ala Val Leu Leu Val Arg Lys Phe Gln Asn Ser Pro Ala Glu Asp>

490     500     510     520
      *      *      *      *      *
TTC CAG GAG CCG TGC CAG TAT TCC CAG GAG TCC CAG AAG TTC TCC TGC
Phe Gln Glu Pro Cys Gln Tyr Ser Gln Glu Ser Gln Lys Phe Ser Cys>

530     540     550     560     570
      *      *      *      *      *
CAG TTA GCA GTC CCG GAG GGA GAC AGC TCT TTC TAC ATA GTG TCC ATG
Gln Leu Ala Val Pro Glu Gly Asp Ser Ser Phe Tyr Ile Val Ser Met>

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Fig.25B.

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580      590      600      610      620
*      *      *      *      *
TGC GTC GCC AGT AGT GTC GGG AGC AAG TTC AGC AAA ACT CAA ACC TTT
Cys Val Ala Ser Ser Val Gly Ser Lys Phe Ser Lys Thr Gln Thr Phe>

630      640      650      660      670
*      *      *      *      *
CAG GGT TGT GGA ATC TTG CAG CCT GAT CCG CCT GCC AAC ATC ACA GTC
Gln Gly Cys Gly Ile Leu Gln Pro Asp Pro Pro Ala Asn Ile Thr Val>

680      690      700      710      720
*      *      *      *      *
ACT GCC GTG GCC AGA AAC CCC CGC TGG CTC AGT GTC ACC TGG CAA GAC
Thr Ala Val Ala Arg Asn Pro Arg Trp Leu Ser Val Thr Trp Gln Asp>

730      740      750      760
*      *      *      *
CCC CAC TCC TGG AAC TCA TCT TTC TAC AGA CTA CGG TTT GAG CTC AGA
Pro His Ser Trp Asn Ser Ser Phe Tyr Arg Leu Arg Phe Glu Leu Arg>

770      780      790      800      810
*      *      *      *      *
TAT CGG GCT GAA CGG TCA AAG ACA TTC ACA ACA TGG ATG GTC AAG GAC
Tyr Arg Ala Glu Arg Ser Lys Thr Phe Thr Thr Trp Met Val Lys Asp>

820      830      840      850      860
*      *      *      *      *
CTC CAG CAT CAC TGT GTC ATC CAC GAC GCC TGG AGC GGC CTG AGG CAC
Leu Gln His His Cys Val Ile His Asp Ala Trp Ser Gly Leu Arg His>

870      880      890      900      910
*      *      *      *      *
GTG GTG CAG CTT CGT GCC CAG GAG GAG TTC GGG CAA GGC GAG TGG AGC
Val Val Gln Leu Arg Ala Gln Glu Glu Phe Gly Gln Gly Glu Trp Ser>

920      930      940      950      960
*      *      *      *      *
GAG TGG AGC CCG GAG GCC ATG GGC ACG CCT TGG ACA GAA TCG CGA TCG
Glu Trp Ser Pro Glu Ala Met Gly Thr Pro Trp Thr Glu Ser Arg Ser>

970      980      990      1000
*      *      *      *
CCT CCA GCT GAG AAC GAG GTG TCC ACC CCC ATG GAA CTT CTA GAC CCA
Pro Pro Ala Glu Asn Glu Val Ser Thr Pro Met Glu Leu Leu Asp Pro>

1010      1020      1030      1040      1050
*      *      *      *      *
TGT GGT TAT ATC AGT CCT GAA TCT CCA GTT GTA CAA CTT CAT TCT AAT
Cys Gly Tyr Ile Ser Pro Glu Ser Pro Val Val Gln Leu His Ser Asn>

1060      1070      1080      1090      1100
*      *      *      *      *
TTC ACT GCA GTT TGT GTG CTA AAG GAA AAA TGT ATG GAT TAT TTT CAT
Phe Thr Ala Val Cys Val Leu Lys Glu Lys Cys Met Asp Tyr Phe His>

1110      1120      1130      1140      1150
*      *      *      *      *
GTA AAT GCT AAT TAC ATT GTC TGG AAA ACA AAC CAT TTT ACT ATT CCT
Val Asn Ala Asn Tyr Ile Val Trp Lys Thr Asn His Phe Thr Ile Pro>

1160      1170      1180      1190      1200
*      *      *      *      *

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Fig.25C.

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AAG GAG CAA TAT ACT ATC ATA AAC AGA ACA GCA TCC AGT GTC ACC TTT
Lys Glu Gln Tyr Thr Ile Ile Asn Arg Thr Ala Ser Ser Val Thr Phe>

      1210      1220      1230      1240
      *      *      *      *      *      *
ACA GAT ATA GCT TCA TTA AAT ATT CAG CTC ACT TGC AAC ATT CTT ACA
Thr Asp Ile Ala Ser Leu Asn Ile Gln Leu Thr Cys Asn Ile Leu Thr>

1250      1260      1270      1280      1290
*      *      *      *      *      *
TTC GGA CAG CTT GAA CAG AAT GTT TAT GGA ATC ACA ATA ATT TCA GGC
Phe Gly Gln Leu Glu Gln Asn Val Tyr Gly Ile Thr Ile Ile Ser Gly>

      1300      1310      1320      1330      1340
      *      *      *      *      *
TTG CCT CCA GAA AAA CCT AAA AAT TTG AGT TGC ATT GTG AAC GAG GGG
Leu Pro Pro Glu Lys Pro Lys Asn Leu Ser Cys Ile Val Asn Glu Gly>

      1350      1360      1370      1380      1390
      *      *      *      *      *
AAG AAA ATG AGG TGT GAG TGG GAT GGT GGA AGG GAA ACA CAC TTG GAG
Lys Lys Met Arg Cys Glu Trp Asp Gly Gly Arg Glu Thr His Leu Glu>

      1400      1410      1420      1430      1440
      *      *      *      *      *
ACA AAC TTC ACT TTA AAA TCT GAA TGG GCA ACA CAC AAG TTT GCT GAT
Thr Asn Phe Thr Leu Lys Ser Glu Trp Ala Thr His Lys Phe Ala Asp>

      1450      1460      1470      1480
      *      *      *      *      *
TGC AAA GCA AAA CGT GAC ACC CCC ACC TCA TGC ACT GTT GAT TAT TCT
Cys Lys Ala Lys Arg Asp Thr Pro Thr Ser Cys Thr Val Asp Tyr Ser>

1490      1500      1510      1520      1530
*      *      *      *      *
ACT GTG TAT TTT GTC AAC ATT GAA GTC TGG GTA GAA GCA GAG AAT GCC
Thr Val Tyr Phe Val Asn Ile Glu Val Trp Val Glu Ala Glu Asn Ala>

      1540      1550      1560      1570      1580
      *      *      *      *      *
CTT GGG AAG GTT ACA TCA GAT CAT ATC AAT TTT GAT CCT GTA TAT AAA
Leu Gly Lys Val Thr Ser Asp His Ile Asn Phe Asp Pro Val Tyr Lys>

      1590      1600      1610      1620      1630
      *      *      *      *      *
GTG AAG CCC AAT CCG CCA CAT AAT TTA TCA GTG ATC AAC TCA GAG GAA
Val Lys Pro Asn Pro Pro His Asn Leu Ser Val Ile Asn Ser Glu Glu>

      1640      1650      1660      1670      1680
      *      *      *      *      *
CTG TCT AGT ATC TTA AAA TTG ACA TGG ACC AAC CCA AGT ATT AAG AGT
Leu Ser Ser Ile Leu Lys Leu Thr Trp Thr Asn Pro Ser Ile Lys Ser>

      1690      1700      1710      1720
      *      *      *      *      *
GTT ATA ATA CTA AAA TAT AAC ATT CAA TAT AGG ACC AAA GAT GCC TCA
Val Ile Ile Leu Lys Tyr Asn Ile Gln Tyr Arg Thr Lys Asp Ala Ser>

1730      1740      1750      1760      1770
*      *      *      *      *
ACT TGG AGC CAG ATT CCT CCT GAA GAC ACA GCA TCC ACC CGA TCT TCA
Thr Trp Ser Gln Ile Pro Pro Glu Asp Thr Ala Ser Thr Arg Ser Ser>

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Fig.25D.

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1780      1790      1800      1810      1820
*          *          *          *          *
TTC ACT GTC CAA GAC CTT AAA CCT TTT ACA GAA TAT GTG TTT AGG ATT
Phe Thr Val Gln Asp Leu Lys Pro Phe Thr Glu Tyr Val Phe Arg Ile>

1830      1840      1850      1860      1870
*          *          *          *          *
CGC TGT ATG AAG GAA GAT GGT AAG GGA TAC TGG AGT GAC TGG AGT GAA
Arg Cys Met Lys Glu Asp Gly Lys Gly Tyr Trp Ser Asp Trp Ser Glu>

1880      1890      1900      1910      1920
*          *          *          *          *
GAA GCA AGT GGG ATC ACC TAT GAA GAT AGA CCA TCT AAA GCA CCA AGT
Glu Ala Ser Gly Ile Thr Tyr Glu Asp Arg Pro Ser Lys Ala Pro Ser>

1930      1940      1950      1960
*          *          *          *          *
TTC TGG TAT AAA ATA GAT CCA TCC CAT ACT CAA GGC TAC AGA ACT GTA
Phe Trp Tyr Lys Ile Asp Pro Ser His Thr Gln Gly Tyr Arg Thr Val>

1970      1980      1990      2000      2010
*          *          *          *          *
CAA CTC GTG TGG AAG ACA TTG CCT CCT TTT GAA GCC AAT GGA AAA ATC
Gln Leu Val Trp Lys Thr Leu Pro Pro Phe Glu Ala Asn Gly Lys Ile>

2020      2030      2040      2050      2060
*          *          *          *          *
TTG GAT TAT GAA GTG ACT CTC ACA AGA TGG AAA TCA CAT TTA CAA AAT
Leu Asp Tyr Glu Val Thr Leu Thr Arg Trp Lys Ser His Leu Gln Asn>

2070      2080      2090      2100      2110
*          *          *          *          *
TAC ACA GTT AAT GCC ACA AAA CTG ACA GTA AAT CTC ACA AAT GAT CGC
Tyr Thr Val Asn Ala Thr Lys Leu Thr Val Asn Leu Thr Asn Asp Arg>

2120      2130      2140      2150      2160
*          *          *          *          *
TAT CTA GCA ACC CTA ACA GTA AGA AAT CTT GTT GGC AAA TCA GAT GCA
Tyr Leu Ala Thr Leu Thr Val Arg Asn Leu Val Gly Lys Ser Asp Ala>

2170      2180      2190      2200
*          *          *          *          *
GCT GTT TTA ACT ATC CCT GCC TGT GAC TTT CAA GCT ACT CAC CCT GTA
Ala Val Leu Thr Ile Pro Ala Cys Asp Phe Gln Ala Thr His Pro Val>

2210      2220      2230      2240      2250
*          *          *          *          *
ATG GAT CTT AAA GCA TTC CCC AAA GAT AAC ATG CTT TGG GTG GAA TGG
Met Asp Leu Lys Ala Phe Pro Lys Asp Asn Met Leu Trp Val Glu Trp>

2260      2270      2280      2290      2300
*          *          *          *          *
ACT ACT CCA AGG GAA TCT GTA AAG AAA TAT ATA CTT GAG TGG TGT GTG
Thr Thr Pro Arg Glu Ser Val Lys Lys Tyr Ile Leu Glu Trp Cys Val>

2310      2320      2330      2340      2350
*          *          *          *          *
TTA TCA GAT AAA GCA CCC TGT ATC ACA GAC TGG CAA CAA GAA GAT GGT
Leu Ser Asp Lys Ala Pro Cys Ile Thr Asp Trp Gln Gln Glu Asp Gly>

2360      2370      2380      2390      2400

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Fig.25E.

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*      *      *      *      *      *      *      *      *
ACC GTG CAT CGC ACC TAT TTA AGA GGG AAC TTA GCA GAG AGC AAA TGC
Thr Val His Arg Thr Tyr Leu Arg Gly Asn Leu Ala Glu Ser Lys Cys>

      2410      2420      2430      2440
*      *      *      *      *      *      *      *
TAT TTG ATA ACA GTT ACT CCA GTA TAT GCT GAT GGA CCA GGA AGC CCT
Tyr Leu Ile Thr Val Thr Pro Val Tyr Ala Asp Gly Pro Gly Ser Pro>

2450      2460      2470      2480      2490
*      *      *      *      *      *      *      *
GAA TCC ATA AAG GCA TAC CTT AAA CAA GCT CCA CCT TCC AAA GGA CCT
Glu Ser Ile Lys Ala Tyr Leu Lys Gln Ala Pro Pro Ser Lys Gly Pro>

      2500      2510      2520      2530      2540
*      *      *      *      *      *      *      *
ACT GTT CGG ACA AAA AAA GTA GGG AAA AAC GAA GCT GTC TTA GAG TGG
Thr Val Arg Thr Lys Lys Val Gly Lys Asn Glu Ala Val Leu Glu Trp>

      2550      2560      2570      2580      2590
*      *      *      *      *      *      *      *
GAC CAA CTT CCT GTT GAT GTT CAG AAT GGA TTT ATC AGA AAT TAT ACT
Asp Gln Leu Pro Val Asp Val Gln Asn Gly Phe Ile Arg Asn Tyr Thr>

      2600      2610      2620      2630      2640
*      *      *      *      *      *      *      *
ATA TTT TAT AGA ACC ATC ATT GGA AAT GAA ACT GCT GTG AAT GTG GAT
Ile Phe Tyr Arg Thr Ile Ile Gly Asn Glu Thr Ala Val Asn Val Asp>

      2650      2660      2670      2680
*      *      *      *      *      *      *      *
TCT TCC CAC ACA GAA TAT ACA TTG TCC TCT TTG ACT AGT GAC ACA TTG
Ser Ser His Thr Glu Tyr Thr Leu Ser Ser Leu Thr Ser Asp Thr Leu>

2690      2700      2710      2720      2730
*      *      *      *      *      *      *      *
TAC ATG GTA CGA ATG GCA GCA TAC ACA GAT GAA GGT GGG AAG GAT GGT
Tyr Met Val Arg Met Ala Ala Tyr Thr Asp Glu Gly Gly Lys Asp Gly>

      2740      2750      2760      2770      2780
*      *      *      *      *      *      *      *
CCA GAA TTC ACT TTT ACT ACC CCA AAG TTT GCT CAA GGA GAA ATT GAA
Pro Glu Phe Thr Phe Thr Thr Pro Lys Phe Ala Gln Gly Glu Ile Glu>

      2790      2800      2810      2820      2830
*      *      *      *      *      *      *      *
TCC GGG GGC GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA
Ser Gly Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu>

      2840      2850      2860      2870      2880
*      *      *      *      *      *      *      *
CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp>

      2890      2900      2910      2920
*      *      *      *      *      *      *      *
ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp>

2930      2940      2950      2960      2970
*      *      *      *      *      *      *      *
GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC

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Fig.25F.

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Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly>
 2980 2990 3000 3010 3020
 * * * * *
 GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC
 Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn>
 3030 3040 3050 3060 3070
 * * * * *
 AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG
 Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp>
 3080 3090 3100 3110 3120
 * * * * *
 CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA
 Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro>
 3130 3140 3150 3160
 * * * * *
 GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA
 Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu>
 3170 3180 3190 3200 3210
 * * * * *
 CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC
 Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn>
 3220 3230 3240 3250 3260
 * * * * *
 CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC
 Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile>
 3270 3280 3290 3300 3310
 * * * * *
 GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC
 Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr>
 3320 3330 3340 3350 3360
 * * * * *
 ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG
 Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys>
 3370 3380 3390 3400
 * * * *
 CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC
 Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys>
 3410 3420 3430 3440 3450
 * * * * *
 TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC
 Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu>
 3460 3470
 * * *
 TCC CTG TCT CCG GGT AAA TGA
 Ser Leu Ser Pro Gly Lys ***>

Fig.26A.

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      10      20      30      40
      *      *      *      *
ATG GTG CTT CTG TGG TGT GTA GTG AGT CTC TAC TTT TAT GGA ATC CTG
Met Val Leu Leu Trp Cys Val Val Ser Leu Tyr Phe Tyr Gly Ile Leu>

50      60      70      80      90
      *      *      *      *      *
CAA AGT GAT GCC TCA GAA CGC TGC GAT GAC TGG GGA CTA GAC ACC ATG
Gln Ser Asp Ala Ser Glu Arg Cys Asp Asp Trp Gly Leu Asp Thr Met>

100     110     120     130     140
      *      *      *      *      *
AGG CAA ATC CAA GTG TTT GAA GAT GAG CCA GCT CGC ATC AAG TGC CCA
Arg Gln Ile Gln Val Phe Glu Asp Glu Pro Ala Arg Ile Lys Cys Pro>

150     160     170     180     190
      *      *      *      *      *
CTC TTT GAA CAC TTC TTG AAA TTC AAC TAC AGC ACA GCC CAT TCA GCT
Leu Phe Glu His Phe Leu Lys Phe Asn Tyr Ser Thr Ala His Ser Ala>

200     210     220     230     240
      *      *      *      *      *
GGC CTT ACT CTG ATC TGG TAT TGG ACT AGG CAG GAC CGG GAC CTT GAG
Gly Leu Thr Leu Ile Trp Tyr Trp Thr Arg Gln Asp Arg Asp Leu Glu>

250     260     270     280
      *      *      *      *      *
GAG CCA ATT AAC TTC CGC CTC CCC GAG AAC CGC ATT AGT AAG GAG AAA
Glu Pro Ile Asn Phe Arg Leu Pro Glu Asn Arg Ile Ser Lys Glu Lys>

290     300     310     320     330
      *      *      *      *      *
GAT GTG CTG TGG TTC CGG CCC ACT CTC CTC AAT GAC ACT GGC AAC TAT
Asp Val Leu Trp Phe Arg Pro Thr Leu Leu Asn Asp Thr Gly Asn Tyr>

340     350     360     370     380
      *      *      *      *      *
ACC TGC ATG TTA AGG AAC ACT ACA TAT TGC AGC AAA GTT GCA TTT CCC
Thr Cys Met Leu Arg Asn Thr Thr Tyr Cys Ser Lys Val Ala Phe Pro>

390     400     410     420     430
      *      *      *      *      *
TTG GAA GTT GTT CAA AAA GAC AGC TGT TTC AAT TCC CCC ATG AAA CTC
Leu Glu Val Val Gln Lys Asp Ser Cys Phe Asn Ser Pro Met Lys Leu>

440     450     460     470     480
      *      *      *      *      *
CCA GTG CAT AAA CTG TAT ATA GAA TAT GGC ATT CAG AGG ATC ACT TGT
Pro Val His Lys Leu Tyr Ile Glu Tyr Gly Ile Gln Arg Ile Thr Cys>

490     500     510     520
      *      *      *      *      *
CCA AAT GTA GAT GGA TAT TTT CCT TCC AGT GTC AAA CCG ACT ATC ACT
Pro Asn Val Asp Gly Tyr Phe Pro Ser Ser Val Lys Pro Thr Ile Thr>

530     540     550     560     570
      *      *      *      *      *
TGG TAT ATG GGC TGT TAT AAA ATA CAG AAT TTT AAT AAT GTA ATA CCC
Trp Tyr Met Gly Cys Tyr Lys Ile Gln Asn Phe Asn Asn Val Ile Pro>

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Fig.26B.

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580      590      600      610      620
*      *      *      *      *
GAA GGT ATG AAC TTG AGT TTC CTC ATT GCC TTA ATT TCA AAT AAT GGA
Glu Gly Met Asn Leu Ser Phe Leu Ile Ala Leu Ile Ser Asn Asn Gly>

630      640      650      660      670
*      *      *      *      *
AAT TAC ACA TGT GTT GTT ACA TAT CCA GAA AAT GGA CGT ACG TTT CAT
Asn Tyr Thr Cys Val Val Thr Tyr Pro Glu Asn Gly Arg Thr Phe His>

680      690      700      710      720
*      *      *      *      *
CTC ACC AGG ACT CTG ACT GTA AAG GTA GTA GGC TCT CCA AAA AAT GCA
Leu Thr Arg Thr Leu Thr Val Lys Val Val Gly Ser Pro Lys Asn Ala>

730      740      750      760
*      *      *      *      *
GTG CCC CCT GTG ATC CAT TCA CCT AAT GAT CAT GTG GTC TAT GAG AAA
Val Pro Pro Val Ile His Ser Pro Asn Asp His Val Val Tyr Glu Lys>

770      780      790      800      810
*      *      *      *      *
GAA CCA GGA GAG GAG CTA CTC ATT CCC TGT ACG GTC TAT TTT AGT TTT
Glu Pro Gly Glu Glu Leu Leu Ile Pro Cys Thr Val Tyr Phe Ser Phe>

820      830      840      850      860
*      *      *      *      *
CTG ATG GAT TCT CGC AAT GAG GTT TGG TGG ACC ATT GAT GGA AAA AAA
Leu Met Asp Ser Arg Asn Glu Val Trp Trp Thr Ile Asp Gly Lys Lys>

870      880      890      900      910
*      *      *      *      *
CCT GAT GAC ATC ACT ATT GAT GTC ACC ATT AAC GAA AGT ATA AGT CAT
Pro Asp Asp Ile Thr Ile Asp Val Thr Ile Asn Glu Ser Ile Ser His>

920      930      940      950      960
*      *      *      *      *
AGT AGA ACA GAA GAT GAA ACA AGA ACT CAG ATT TTG AGC ATC AAG AAA
Ser Arg Thr Glu Asp Glu Thr Arg Thr Gln Ile Leu Ser Ile Lys Lys>

970      980      990      1000
*      *      *      *      *
GTT ACC TCT GAG GAT CTC AAG CGC AGC TAT GTC TGT CAT GCT AGA AGT
Val Thr Ser Glu Asp Leu Lys Arg Ser Tyr Val Cys His Ala Arg Ser>

1010      1020      1030      1040      1050
*      *      *      *      *
GCC AAA GGC GAA GTT GCC AAA GCA GCC AAG GTG AAG CAG AAA GTG CCA
Ala Lys Gly Glu Val Ala Lys Ala Ala Lys Val Lys Gln Lys Val Pro>

1060      1070      1080      1090      1100
*      *      *      *      *
GCT CCA AGA TAC ACA GTG TCC GGT GGC GCG CCT ATG CTG AGC GAG GCT
Ala Pro Arg Tyr Thr Val Ser Gly Gly Ala Pro Met Leu Ser Glu Ala>

1110      1120      1130      1140      1150
*      *      *      *      *
GAT AAA TGC AAG GAA CGT GAA GAA AAA ATA ATT TTA GTG TCA TCT GCA
Asp Lys Cys Lys Glu Arg Glu Glu Lys Ile Ile Leu Val Ser Ser Ala>

1160      1170      1180      1190      1200
*      *      *      *      *

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Fig.26C.

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AAT GAA ATT GAT GTT CGT CCC TGT CCT CTT AAC CCA AAT GAA CAC AAA
Asn Glu Ile Asp Val Arg Pro Cys Pro Leu Asn Pro Asn Glu His Lys>

      1210      1220      1230      1240
      *      *      *      *      *      *
GGC ACT ATA ACT TGG TAT AAG GAT GAC AGC AAG ACA CCT GTA TCT ACA
Gly Thr Ile Thr Trp Tyr Lys Asp Asp Ser Lys Thr Pro Val Ser Thr>

1250      1260      1270      1280      1290
      *      *      *      *      *      *
GAA CAA GCC TCC AGG ATT CAT CAA CAC AAA GAG AAA CTT TGG TTT GTT
Glu Gln Ala Ser Arg Ile His Gln His Lys Glu Lys Leu Trp Phe Val>

      1300      1310      1320      1330      1340
      *      *      *      *      *      *
CCT GCT AAG GTG GAG GAT TCA GGA CAT TAC TAT TGC GTG GTA AGA AAT
Pro Ala Lys Val Glu Asp Ser Gly His Tyr Tyr Cys Val Val Arg Asn>

      1350      1360      1370      1380      1390
      *      *      *      *      *      *
TCA TCT TAC TGC CTC AGA ATT AAA ATA AGT GCA AAA TTT GTG GAG AAT
Ser Ser Tyr Cys Leu Arg Ile Lys Ile Ser Ala Lys Phe Val Glu Asn>

      1400      1410      1420      1430      1440
      *      *      *      *      *      *
GAG CCT AAC TTA TGT TAT AAT GCA CAA GCC ATA TTT AAG CAG AAA CTA
Glu Pro Asn Leu Cys Tyr Asn Ala Gln Ala Ile Phe Lys Gln Lys Leu>

      1450      1460      1470      1480
      *      *      *      *      *      *
CCC GTT GCA GGA GAC GGA GGA CTT GTG TGC CCT TAT ATG GAG TTT TTT
Pro Val Ala Gly Asp Gly Gly Leu Val Cys Pro Tyr Met Glu Phe Phe>

1490      1500      1510      1520      1530
      *      *      *      *      *      *
AAA AAT GAA AAT AAT GAG TTA CCT AAA TTA CAG TGG TAT AAG GAT TGC
Lys Asn Glu Asn Asn Glu Leu Pro Lys Leu Gln Trp Tyr Lys Asp Cys>

      1540      1550      1560      1570      1580
      *      *      *      *      *      *
AAA CCT CTA CTT CTT GAC AAT ATA CAC TTT AGT GGA GTC AAA GAT AGG
Lys Pro Leu Leu Leu Asp Asn Ile His Phe Ser Gly Val Lys Asp Arg>

      1590      1600      1610      1620      1630
      *      *      *      *      *      *
CTC ATC GTG ATG AAT GTG GCT GAA AAG CAT AGA GGG AAC TAT ACT TGT
Leu Ile Val Met Asn Val Ala Glu Lys His Arg Gly Asn Tyr Thr Cys>

      1640      1650      1660      1670      1680
      *      *      *      *      *      *
CAT GCA TCC TAC ACA TAC TTG GGC AAG CAA TAT CCT ATT ACC CGG GTA
His Ala Ser Tyr Thr Tyr Leu Gly Lys Gln Tyr Pro Ile Thr Arg Val>

      1690      1700      1710      1720
      *      *      *      *      *      *
ATA GAA TTT ATT ACT CTA GAG GAA AAC AAA CCC ACA AGG CCT GTG ATT
Ile Glu Phe Ile Thr Leu Glu Glu Asn Lys Pro Thr Arg Pro Val Ile>

1730      1740      1750      1760      1770
      *      *      *      *      *      *
GTG AGC CCA GCT AAT GAG ACA ATG GAA GTA GAC TTG GGA TCC CAG ATA
Val Ser Pro Ala Asn Glu Thr Met Glu Val Asp Leu Gly Ser Gln Ile>

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Fig.26D.

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1780      1790      1800      1810      1820
*          *          *          *          *
CAA TTG ATC TGT AAT GTC ACC GGC CAG TTG AGT GAC ATT GCT TAC TGG
Gln Leu Ile Cys Asn Val Thr Gly Gln Leu Ser Asp Ile Ala Tyr Trp>

      1830      1840      1850      1860      1870
*          *          *          *          *
AAG TGG AAT GGG TCA GTA ATT GAT GAA GAT GAC CCA GTG CTA GGG GAA
Lys Trp Asn Gly Ser Val Ile Asp Glu Asp Asp Pro Val Leu Gly Glu>

      1880      1890      1900      1910      1920
*          *          *          *          *
GAC TAT TAC AGT GTG GAA AAT CCT GCA AAC AAA AGA AGG AGT ACC CTC
Asp Tyr Tyr Ser Val Glu Asn Pro Ala Asn Lys Arg Arg Ser Thr Leu>

      1930      1940      1950      1960
*          *          *          *          *
ATC ACA GTG CTT AAT ATA TCG GAA ATT GAG AGT AGA TTT TAT AAA CAT
Ile Thr Val Leu Asn Ile Ser Glu Ile Glu Ser Arg Phe Tyr Lys His>

1970      1980      1990      2000      2010
*          *          *          *          *
CCA TTT ACC TGT TTT GCC AAG AAT ACA CAT GGT ATA GAT GCA GCA TAT
Pro Phe Thr Cys Phe Ala Lys Asn Thr His Gly Ile Asp Ala Ala Tyr>

      2020      2030      2040      2050      2060
*          *          *          *          *
ATC CAG TTA ATA TAT CCA GTC ACT AAT TCC GGA GAC AAA ACT CAC ACA
Ile Gln Leu Ile Tyr Pro Val Thr Asn Ser Gly Asp Lys Thr His Thr>

      2070      2080      2090      2100      2110
*          *          *          *          *
TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC
Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe>

      2120      2130      2140      2150      2160
*          *          *          *          *
CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro>

      2170      2180      2190      2200
*          *          *          *          *
GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC
Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val>

2210      2220      2230      2240      2250
*          *          *          *          *
AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA
Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr>

      2260      2270      2280      2290      2300
*          *          *          *          *
AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val>

      2310      2320      2330      2340      2350
*          *          *          *          *
CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys>

      2360      2370      2380      2390      2400

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Fig.26E.

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      *      *      *      *      *      *      *      *      *
AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC
Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser>

      2410      2420      2430      2440
      *      *      *      *      *      *      *      *
AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro>

2450      2460      2470      2480      2490
      *      *      *      *      *      *      *      *
TCC CGG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC
Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val>

      2500      2510      2520      2530      2540
      *      *      *      *      *      *      *      *
AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly>

      2550      2560      2570      2580      2590
      *      *      *      *      *      *      *      *
CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp>

      2600      2610      2620      2630      2640
      *      *      *      *      *      *      *      *
GGC TCC TTC TTC CTC TAT AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp>

      2650      2660      2670      2680
      *      *      *      *      *      *      *      *
CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His>

2690      2700      2710      2720      2730
      *      *      *      *      *      *      *      *
AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys ***>

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Fig.27.

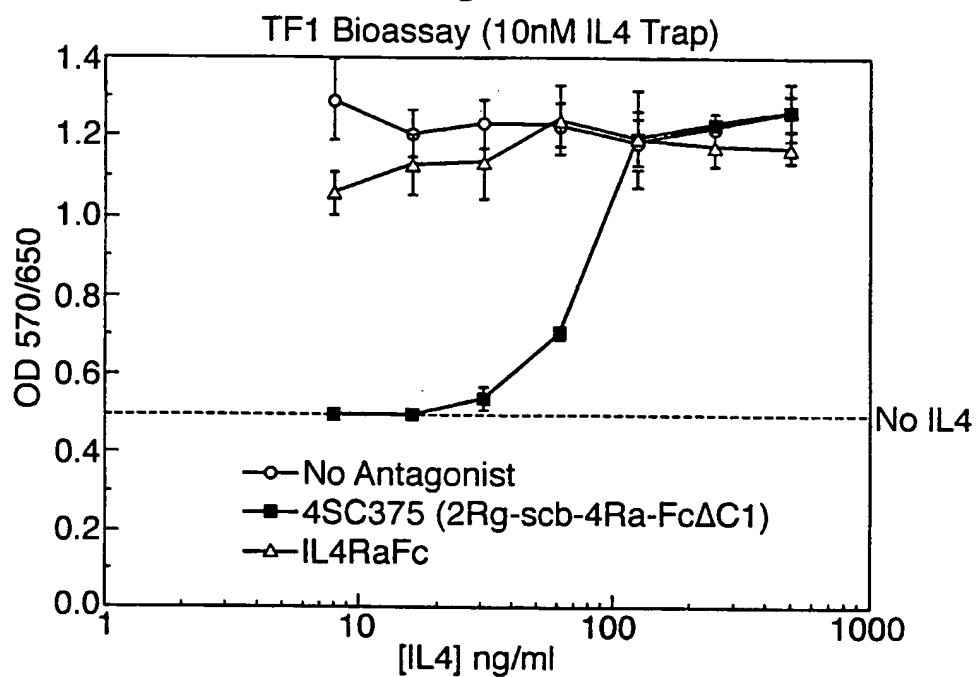
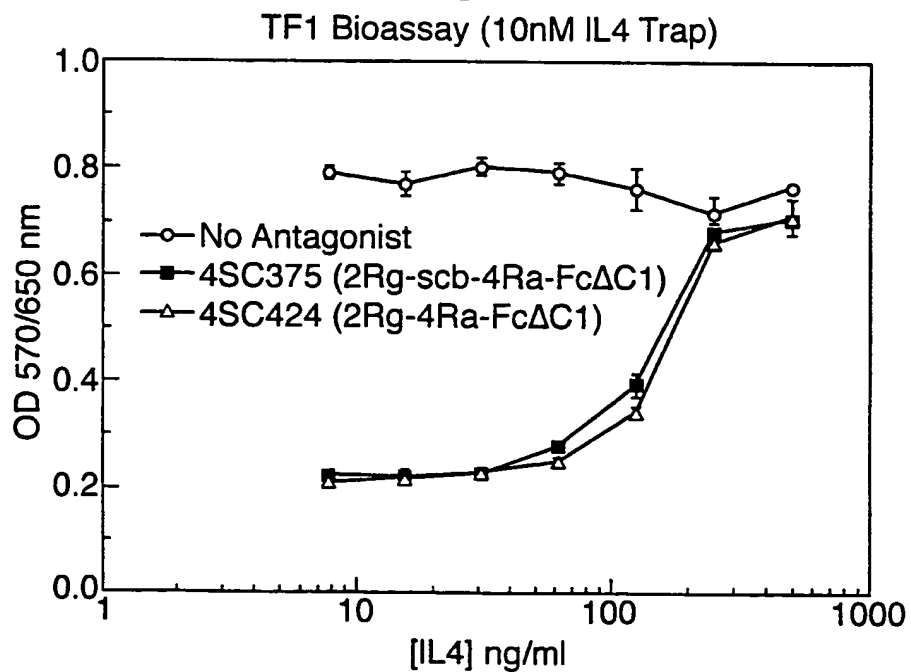


Fig.28.



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Fig.29.

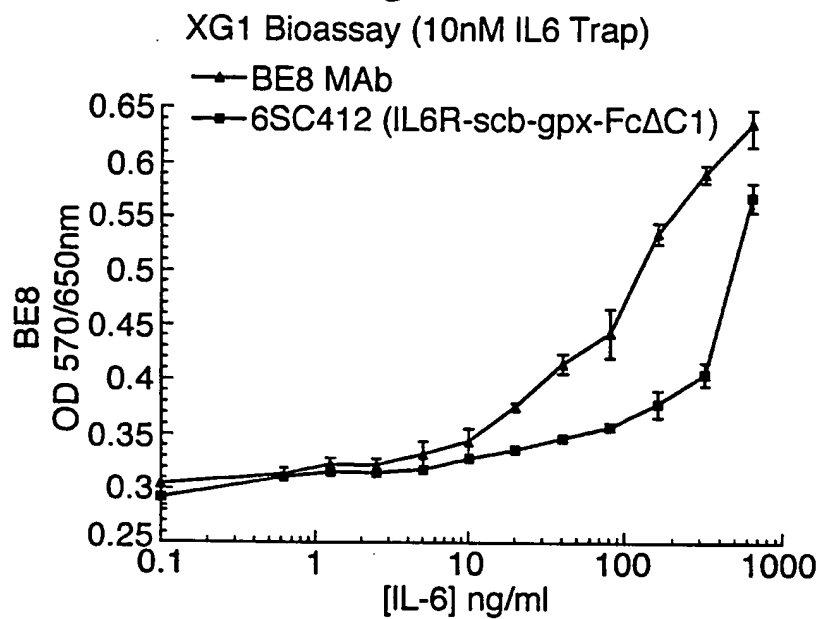
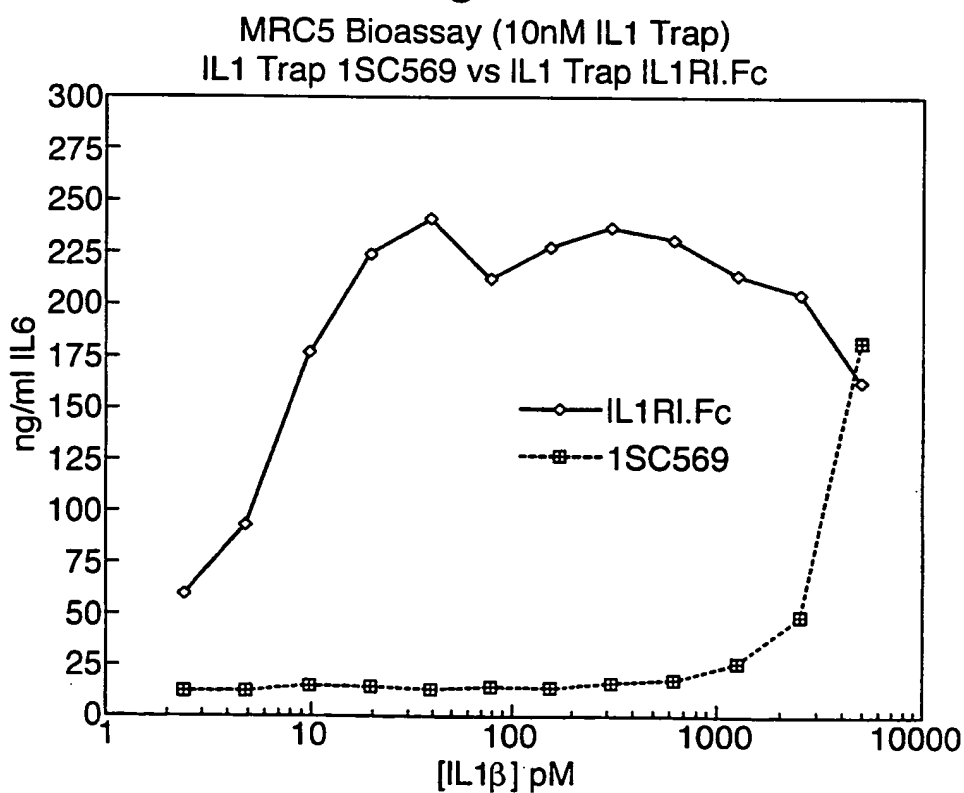


Fig.30.



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Fig.31A.

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      10      20      30      40
      *      *      *      *      *
ATG GTG TGG CTT TGC TCT GGG CTC CTG TTC CCT GTG AGC TGC CTG GTC
TAC CAC ACC GAA ACG AGA CCC GAG GAC AAG GGA CAC TCG ACG GAC CAG
Met Val Trp Leu Cys Ser Gly Leu Leu Phe Pro Val Ser Cys Leu Val>

50      60      70      80      90
      *      *      *      *      *
CTG CTG CAG GTG GCA AGC TCT GGG AAC ATG AAG GTC TTG CAG GAG CCC
GAC GAC GTC CAC CGT TCG AGA CCC TTG TAC TTC CAG AAC GTC CTC GGG
Leu Leu Gln Val Ala Ser Ser Gly Asn Met Lys Val Leu Gln Glu Pro>

100      110      120      130      140
      *      *      *      *      *
ACC TGC GTC TCC GAC TAC ATG AGC ATC TCT ACT TGC GAG TGG AAG ATG
TGG ACG CAG AGG CTG ATG TAC TCG TAG AGA TGA ACG CTC ACC TTC TAC
Thr Cys Val Ser Asp Tyr Met Ser Ile Ser Thr Cys Glu Trp Lys Met>

150      160      170      180      190
      *      *      *      *      *
AAT GGT CCC ACC AAT TGC AGC ACC GAG CTC CGC CTG TTG TAC CAG CTG
TTA CCA GGG TGG TTA ACG TCG TGG CTC GAG GCG GAC AAC ATG GTC GAC
Asn Gly Pro Thr Asn Cys Ser Thr Glu Leu Arg Leu Leu Tyr Gln Leu>

200      210      220      230      240
      *      *      *      *      *
GTT TTT CTG CTC TCC GAA GCC CAC ACG TGT ATC CCT GAG AAC AAC GGA
CAA AAA GAC GAG AGG CTT CGG GTG TGC ACA TAG GGA CTC TTG TTG CCT
Val Phe Leu Leu Ser Glu Ala His Thr Cys Ile Pro Glu Asn Asn Gly>

250      260      270      280
      *      *      *      *      *
GGC GCG GGG TGC GTG TGC CAC CTG CTC ATG GAT GAC GTG GTC AGT GCG
CCG CGC CCC ACG CAC ACG GTG GAC GAG TAC CTA CTG CAC CAG TCA CGC
Gly Ala Gly Cys Val Cys His Leu Leu Met Asp Asp Val Val Ser Ala>

290      300      310      320      330
      *      *      *      *      *
GAT AAC TAT ACA CTG GAC CTG TGG GCT GGG CAG CAG CTG CTG TGG AAG
CTA TTG ATA TGT GAC CTG GAC ACC CGA CCC GTC GTC GAC GAC ACC TTC
Asp Asn Tyr Thr Leu Asp Leu Trp Ala Gly Gln Gln Leu Leu Trp Lys>

340      350      360      370      380
      *      *      *      *      *
GGC TCC TTC AAG CCC AGC GAG CAT GTG AAA CCC AGG GCC CCA GGA AAC
CCG AGG AAG TTC GGG TCG CTC GTA CAC TTT GGG TCC CGG GGT CCT TTG
Gly Ser Phe Lys Pro Ser Glu His Val Lys Pro Arg Ala Pro Gly Asn>

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Fig.31B.

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      390          400          410          420          430
      *           *           *           *           *
CTG ACA GTT CAC ACC AAT GTC TCC GAC ACT CTG CTG CTG ACC TGG AGC
GAC TGT CAA GTG TGG TTA CAG AGG CTG TGA GAC GAC GAC TGG ACC TCG
Leu Thr Val His Thr Asn Val Ser Asp Thr Leu Leu Leu Thr Trp Ser>

      440          450          460          470          480
      *           *           *           *           *
AAC CCG TAT CCC CCT GAC AAT TAC CTG TAT AAT CAT CTC ACC TAT GCA
TTG GGC ATA GGG GGA CTG TTA ATG GAC ATA TTA GTA GAG TGG ATA CGT
Asn Pro Tyr Pro Pro Asp Asn Tyr Leu Tyr Asn His Leu Thr Tyr Ala>

      490          500          510          520
      *           *           *           *           *
GTC AAC ATT TGG AGT GAA AAC GAC CCG GCA GAT TTC AGA ATC TAT AAC
CAG TTG TAA ACC TCA CTT TTG CTG GGC CGT CTA AAG TCT TAG ATA TTG
Val Asn Ile Trp Ser Glu Asn Asp Pro Ala Asp Phe Arg Ile Tyr Asn>

530          540          550          560          570
*           *           *           *           *
GTG ACC TAC CTA GAA CCC TCC CTC CGC ATC GCA GCC AGC ACC CTG AAG
CAC TGG ATG GAT CTT GGG AGG GAG GCG TAG CGT CGG TCG TGG GAC TTC
Val Thr Tyr Leu Glu Pro Ser Leu Arg Ile Ala Ala Ser Thr Leu Lys>

      580          590          600          610          620
      *           *           *           *           *
TCT GGG ATT TCC TAC AGG GCA CGG GTG AGG GCC TGG GCT CAG AGC TAT
AGA CCC TAA AGG ATG TCC CGT GCC CAC TCC CGG ACC CGA GTC TCG ATA
Ser Gly Ile Ser Tyr Arg Ala Arg Val Arg Ala Trp Ala Gln Ser Tyr>

      630          640          650          660          670
      *           *           *           *           *
AAC ACC ACC TGG AGT GAG TGG AGC CCC AGC ACC AAG TGG CAC AAC TCC
TTG TGG TGG ACC TCA CTC ACC TCG GGG TCG TGG TTC ACC GTG TTG AGG
Asn Thr Thr Trp Ser Glu Trp Ser Pro Ser Thr Lys Trp His Asn Ser>

      680          690          700          710          720
      *           *           *           *           *
TAC AGG GAG CCC TTC GAG CAG TCC GGT GGG GGC GGG GGC GCC GCG CCT
ATG TCC CTC GGG AAG CTC GTC AGG CCA CCC CCG CCC CCG CGG CGC GGA
Tyr Arg Glu Pro Phe Glu Gln Ser Gly Gly Gly Gly Gly Ala Ala Pro>

      730          740          750          760
      *           *           *           *           *
ACG GAA ACT CAG CCA CCT GTG ACA AAT TTG AGT GTC TCT GTT GAA AAC
TGC CTT TGA GTC GGT GGA CAC TGT TTA AAC TCA CAG AGA CAA CTT TTG
Thr Glu Thr Gln Pro Pro Val Thr Asn Leu Ser Val Ser Val Glu Asn>

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Fig.31C.

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770          780          790          800          810
*           *           *           *           *
CTC TGC ACA GTA ATA TGG ACA TGG AAT CCA CCC GAG GGA GCC AGC TCA
GAG ACG TGT CAT TAT ACC TGT ACC TTA GGT GGG CTC CCT CGG TCG AGT
Leu Cys Thr Val Ile Trp Thr Trp Asn Pro Pro Glu Gly Ala Ser Ser>

      820          830          840          850          860
      *           *           *           *           *
AAT TGT AGT CTA TGG TAT TTT AGT CAT TTT GGC GAC AAA CAA GAT AAG
TTA ACA TCA GAT ACC ATA AAA TCA GTA AAA CCG CTG TTT GTT CTA TTC
Asn Cys Ser Leu Trp Tyr Phe Ser His Phe Gly Asp Lys Gln Asp Lys>

      870          880          890          900          910
*           *           *           *           *
AAA ATA GCT CCG GAA ACT CGT CGT TCA ATA GAA GTA CCC CTG AAT GAG
TTT TAT CGA GGC CTT TGA GCA GCA AGT TAT CTT CAT GGG GAC TTA CTC
Lys Ile Ala Pro Glu Thr Arg Arg Ser Ile Glu Val Pro Leu Asn Glu>

      920          930          940          950          960
*           *           *           *           *
AGG ATT TGT CTG CAA GTG GGG TCC CAG TGT AGC ACC AAT GAG AGT GAG
TCC TAA ACA GAC GTT CAC CCC AGG GTC ACA TCG TGG TTA CTC TCA CTC
Arg Ile Cys Leu Gln Val Gly Ser Gln Cys Ser Thr Asn Glu Ser Glu>

      970          980          990          1000
*           *           *           *           *
AAG CCT AGC ATT TTG GTT GAA AAA TGC ATC TCA CCC CCA GAA GGT GAT
TTC GGA TCG TAA AAC CAA CTT TTT ACG TAG AGT GGG GGT CTT CCA CTA
Lys Pro Ser Ile Leu Val Glu Lys Cys Ile Ser Pro Pro Glu Gly Asp>

1010          1020          1030          1040          1050
*           *           *           *           *
CCT GAG TCT GCT GTG ACT GAG CTT CAA TGC ATT TGG CAC AAC CTG AGC
GGA CTC AGA CGA CAC TGA CTC GAA GTT ACG TAA ACC GTG TTG GAC TCG
Pro Glu Ser Ala Val Thr Glu Leu Gln Cys Ile Trp His Asn Leu Ser>

      1060          1070          1080          1090          1100
*           *           *           *           *
TAC ATG AAG TGT TCT TGG CTC CCT GGA AGG AAT ACC AGT CCC GAC ACT
ATG TAC TTC ACA AGA ACC GAG GGA CCT TCC TTA TGG TCA GGG CTG TGA
Tyr Met Lys Cys Ser Trp Leu Pro Gly Arg Asn Thr Ser Pro Asp Thr>

      1110          1120          1130          1140          1150
*           *           *           *           *
AAC TAT ACT CTC TAC TAT TGG CAC AGA AGC CTG GAA AAA ATT CAT CAA
TTG ATA TGA GAG ATG ATA ACC GTG TCT TCG GAC CTT TTT TAA GTA GTT
Asn Tyr Thr Leu Tyr Tyr Trp His Arg Ser Leu Glu Lys Ile His Gln>

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Fig.31D.

1160	1170	1180	1190	1200
* * *	* *	* *	* *	* *
TGT GAA AAC ATC	TTT AGA GAA GGC	CAA TAC TTT GGT	TGT TCC TTT GAT	
ACA CTT TTG TAG	AAA TCT CTT CCG	GTT ATG AAA CCA	ACA AGG AAA CTA	
Cys Glu Asn Ile	Phe Arg Glu Gly	Gln Tyr Phe Gly	Cys Ser Phe Asp>	

1210	1220	1230	1240
* *	* *	* *	* *
CTG ACC AAA GTG	AAG GAT TCC AGT	TTT GAA CAA CAC	AGT GTC CAA ATA
GAC TGG TTT CAC	TTC CTA AGG TCA	AAA CTT GTT GTG	TCA CAG GTT TAT
Leu Thr Lys Val	Lys Asp Ser Ser	Phe Glu Gln His	Ser Val Gln Ile>

1250	1260	1270	1280	1290
* *	* *	* *	* *	* *
ATG GTC AAG GAT	AAT GCA GGA AAA	ATT AAA CCA TCC	TTC AAT ATA GTG	
TAC CAG TTC CTA	TTA CGT CCT TTT	TAA TTT GGT AGG	AAG TTA TAT CAC	
Met Val Lys Asp	Asn Ala Gly Lys	Ile Lys Pro Ser	Phe Asn Ile Val>	

1300	1310	1320	1330	1340
* *	* *	* *	* *	* *
CCT TTA ACT TCC	CGT GTG AAA CCT	GAT CCT CCA CAT	ATT AAA AAC CTC	
GGA AAT TGA AGG	GCA CAC TTT GGA	CTA GGA GGT GTA	TAA TTT TTG GAG	
Pro Leu Thr Ser	Arg Val Lys Pro	Asp Pro Pro His	Ile Lys Asn Leu>	

1350	1360	1370	1380	1390
* *	* *	* *	* *	* *
TCC TTC CAC AAT	GAT GAC CTA TAT	GTG CAA TGG GAG	AAT CCA CAG AAT	
AGG AAG GTG TTA	CTA CTG GAT ATA	CAC GTT ACC CTC	TTA GGT GTC TTA	
Ser Phe His Asn	Asp Asp Leu Tyr	Val Gln Trp Glu	Asn Pro Gln Asn>	

1400	1410	1420	1430	1440
* *	* *	* *	* *	* *
TTT ATT AGC AGA	TGC CTA TTT TAT	GAA GTA GAA GTC	AAT AAC AGC CAA	
AAA TAA TCG TCT	ACG GAT AAA ATA	CTT CAT CTT CAG	TTA TTG TCG GTT	
Phe Ile Ser Arg	Cys Leu Phe Tyr	Glu Val Glu Val	Asn Asn Ser Gln>	

1450	1460	1470	1480
* *	* *	* *	* *
ACT GAG ACA CAT	AAT GTT TTC TAC	GTC CAA GAG GCT	AAA TGT GAG AAT
TGA CTC TGT GTA	TTA CAA AAG ATG	CAG GTT CTC CGA	TTT ACA CTC TTA
Thr Glu Thr His	Asn Val Phe Tyr	Val Gln Glu Ala	Lys Cys Glu Asn>

1490	1500	1510	1520	1530
* *	* *	* *	* *	* *
CCA GAA TTT GAG	AGA AAT GTG GAG	AAT ACA TCT TGT	TTC ATG GTC CCT	
GGT CTT AAA CTC	TCT TTA CAC CTC	TTA TGT AGA ACA	AAG TAC CAG GGA	
Pro Glu Phe Glu	Arg Asn Val Glu	Asn Thr Ser Cys	Phe Met Val Pro>	

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Fig.31E.

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1540      1550      1560      1570      1580
*        *        *        *        *
GGT GTT CTT CCT GAT ACT TTG AAC ACA GTC AGA ATA AGA GTC AAA ACA
CCA CAA GAA GGA CTA TGA AAC TTG TGT CAG TCT TAT TCT CAG TTT TGT
Gly Val Leu Pro Asp Thr Leu Asn Thr Val Arg Ile Arg Val Lys Thr>

1590      1600      1610      1620      1630
*        *        *        *        *
AAT AAG TTA TGC TAT GAG GAT GAC AAA CTC TGG AGT AAT TGG AGC CAA
TTA TTC AAT ACG ATA CTC CTA CTG TTT GAG ACC TCA TTA ACC TCG GTT
Asn Lys Leu Cys Tyr Glu Asp Asp Lys Leu Trp Ser Asn Trp Ser Gln>

1640      1650      1660      1670      1680
*        *        *        *        *
GAA ATG AGT ATA GGT AAG AAG CGC AAT TCC ACA ACC GGA GAC AAA ACT
CTT TAC TCA TAT CCA TTC TTC GCG TTA AGG TGT TGG CCT CTG TTT TGA
Glu Met Ser Ile Gly Lys Lys Arg Asn Ser Thr Thr Gly Asp Lys Thr>

1690      1700      1710      1720
*        *        *        *        *
CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA
GTG TGT ACG GGT GGC ACG GGT CGT GGA CTT GAG GAC CCC CCT GGC AGT
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser>

1730      1740      1750      1760      1770
*        *        *        *        *
GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG
CAG AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG GAG TAC TAG AGG GCC
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg>

1780      1790      1800      1810      1820
*        *        *        *        *
ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT
TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC TCG GTG CTT CTG GGA
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro>

1830      1840      1850      1860      1870
*        *        *        *        *
GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC
CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA TTA CGG
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala>

1880      1890      1900      1910      1920
*        *        *        *        *
AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC
TTC TGT TTC GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA CAC CAG
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val>

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Fig.31F.

			1930				1940				1950				1960			
	*		*		*		*		*		*		*		*		*	
	AGC	GTC	CTC	ACC	GTC	CTG	CAC	CAG	GAC	TGG	CTG	AAT	GGC	AAG	GAG	TAC		
	TCG	CAG	GAG	TGG	CAG	GAC	GTG	GTC	CTG	ACC	GAC	TTA	CCG	TTC	CTC	ATG		
	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr>		
1970																		
*		*		*		*		*		*		*		*		*		*
AAG	TGC	AAG	GTC	TCC	AAC	AAA	GCC	CTC	CCA	GCC	CCC	ATC	GAG	AAA	ACC			
TTC	ACG	TTC	CAG	AGG	TTG	TTT	CGG	GAG	GGT	CGG	GGG	TAG	CTC	TTT	TGG			
Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr>			
			2020				2030											
	*		*		*		*		*		*		*		*		*	
ATC	TCC	AAA	GCC	AAA	GGG	CAG	CCC	CGA	GAA	CCA	CAG	GTG	TAC	ACC	CTG			
TAG	AGG	TTT	CGG	TTT	CCC	GTC	GGG	GCT	CTT	GGT	GTC	CAC	ATG	TGG	GAC			
Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu>			
			2070				2080											
*	*		*		*		*		*		*		*		*		*	
CCC	CCA	TCC	CGG	GAG	GAG	ATG	ACC	AAG	AAC	CAG	GTC	AGC	CTG	ACC	TGC			
GGG	GGT	AGG	GCC	CTC	CTC	TAC	TGG	TTC	TTG	GTC	CAG	TCG	GAC	TGG	ACG			
Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys>			
			2120				2130											
*	*		*		*		*		*		*		*		*		*	
CTG	GTC	AAA	GGC	TTC	TAT	CCC	AGC	GAC	ATC	GCC	GTG	GAG	TGG	GAG	AGC			
GAC	CAG	TTT	CCG	AAG	ATA	GGG	TCG	CTG	TAG	CGG	CAC	CTC	ACC	CTC	TCG			
Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser>			
			2170				2180											
*	*		*		*		*		*		*		*		*		*	
AAT	GGG	CAG	CCG	GAG	AAC	AAC	TAC	AAG	ACC	ACG	CCT	CCC	GTG	CTG	GAC			
TTA	CCC	GTC	GGC	CTC	TTG	TTG	ATG	TTC	TGG	TGC	GGA	GGG	CAC	GAC	CTG			
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp>			
2210			2220				2230											
*	*		*		*		*		*		*		*		*		*	
TCC	GAC	GGC	TCC	TTC	TTC	CTC	TAT	AGC	AAG	CTC	ACC	GTG	GAC	AAG	AGC			
AGG	CTG	CCG	AGG	AAG	AAG	GAG	ATA	TCG	TTC	GAG	TGG	CAC	CTG	TTC	TCG			
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser>			
			2260				2270											
*	*		*		*		*		*		*		*		*		*	
AGG	TGG	CAG	CAG	GGG	AAC	GTC	TTC	TCA	TGC	TCC	GTG	ATG	CAT	GAG	GCT			
TCC	ACC	GTC	GTC	CCC	TTG	CAG	AAG	AGT	ACG	AGG	CAC	TAC	GTA	CTC	CGA			
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala>			

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Fig.31G.

2310			2320			2330			2340			2350			
*	*		*			*	*		*	*		*	*		
CTG	CAC	AAC	CAC	TAC	ACG	CAG	AAG	AGC	CTC	TCC	CTG	TCT	CCG	GGT	AAA
GAC	GTG	TTG	GTG	ATG	TGC	GTC	TTC	TCG	GAG	AGG	GAC	AGA	GGC	CCA	TTT
Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys>
*															
TGA															
ACT															
***>															

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Fig.32A.

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      10      20      30      40
      *      *      *      *      *
ATG GTG TGG CCG GCG CGG CTC TGC GGG CTG TGG GCG CTG CTG CTC TGC
TAC CAC ACC GGC CGC GCC GAG ACG CCC GAC ACC CGC GAC GAC GAG ACG
Met Val Trp Pro Ala Arg Leu Cys Gly Leu Trp Ala Leu Leu Leu Cys>

50      60      70      80      90
      *      *      *      *      *
GCC GGC GGC GGG GGC GGG GGC GGG GGC GCC GCG CCT ACG GAA ACT CAG
CGG CCG CCG CCC CCG CCC CCG CCC CCG CGG CGC GGA TGC CTT TGA GTC
Ala Gly Gly Gly Gly Gly Gly Gly Gly Ala Ala Pro Thr Glu Thr Gln>

100     110     120     130     140
      *      *      *      *      *
CCA CCT GTG ACA AAT TTG AGT GTC TCT GTT GAA AAC CTC TGC ACA GTA
GGT GGA CAC TGT TTA AAC TCA CAG AGA CAA CTT TTG GAG ACG TGT CAT
Pro Pro Val Thr Asn Leu Ser Val Ser Val Glu Asn Leu Cys Thr Val>

150     160     170     180     190
      *      *      *      *      *
ATA TGG ACA TGG AAT CCA CCC GAG GGA GCC AGC TCA AAT TGT AGT CTA
TAT ACC TGT ACC TTA GGT GGG CTC CCT CGG TCG AGT TTA ACA TCA GAT
Ile Trp Thr Trp Asn Pro Pro Glu Gly Ala Ser Ser Asn Cys Ser Leu>

200     210     220     230     240
      *      *      *      *      *
TGG TAT TTT AGT CAT TTT GGC GAC AAA CAA GAT AAG AAA ATA GCT CCG
ACC ATA AAA TCA GTA AAA CCG CTG TTT GTT CTA TTC TTT TAT CGA GGC
Trp Tyr Phe Ser His Phe Gly Asp Lys Gln Asp Lys Lys Ile Ala Pro>

250     260     270     280
      *      *      *      *      *
GAA ACT CGT CGT TCA ATA GAA GTA CCC CTG AAT GAG AGG ATT TGT CTG
CTT TGA GCA GCA AGT TAT CTT CAT GGG GAC TTA CTC TCC TAA ACA GAC
Glu Thr Arg Arg Ser Ile Glu Val Pro Leu Asn Glu Arg Ile Cys Leu>

290     300     310     320     330
      *      *      *      *      *
CAA GTG GGG TCC CAG TGT AGC ACC AAT GAG AGT GAG AAG CCT AGC ATT
GTT CAC CCC AGG GTC ACA TCG TGG TTA CTC TCA CTC TTC GGA TCG TAA
Gln Val Gly Ser Gln Cys Ser Thr Asn Glu Ser Glu Lys Pro Ser Ile>

340     350     360     370     380
      *      *      *      *      *
TTG GTT GAA AAA TGC ATC TCA CCC CCA GAA GGT GAT CCT GAG TCT GCT
AAC CAA CTT TTT ACG TAG AGT GGG GGT CTT CCA CTA GGA CTC AGA CGA
Leu Val Glu Lys Cys Ile Ser Pro Pro Glu Gly Asp Pro Glu Ser Ala>

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Fig.32B.

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      390      400      410      420      430
      *      *      *      *      *      *      *      *
GTG ACT GAG CTT CAA TGC ATT TGG CAC AAC CTG AGC TAC ATG AAG TGT
CAC TGA CTC GAA GTT ACG TAA ACC GTG TTG GAC TCG ATG TAC TTC ACA
Val Thr Glu Leu Gln Cys Ile Trp His Asn Leu Ser Tyr Met Lys Cys>

      440      450      460      470      480
      *      *      *      *      *      *      *      *
TCT TGG CTC CCT GGA AGG AAT ACC AGT CCC GAC ACT AAC TAT ACT CTC
AGA ACC GAG GGA CCT TCC TTA TGG TCA GGG CTG TGA TTG ATA TGA GAG
Ser Trp Leu Pro Gly Arg Asn Thr Ser Pro Asp Thr Asn Tyr Thr Leu>

      490      500      510      520
      *      *      *      *      *      *      *      *
TAC TAT TGG CAC AGA AGC CTG GAA AAA ATT CAT CAA TGT GAA AAC ATC
ATG ATA ACC GTG TCT TCG GAC CTT TTT TAA GTA GTT ACA CTT TTG TAG
Tyr Tyr Trp His Arg Ser Leu Glu Lys Ile His Gln Cys Glu Asn Ile>

530      540      550      560      570
      *      *      *      *      *      *      *      *
TTT AGA GAA GGC CAA TAC TTT GGT TGT TCC TTT GAT CTG ACC AAA GTG
AAA TCT CTT CCG GTT ATG AAA CCA ACA AGG AAA CTA GAC TGG TTT CAC
Phe Arg Glu Gly Gln Tyr Phe Gly Cys Ser Phe Asp Leu Thr Lys Val>

      580      590      600      610      620
      *      *      *      *      *      *      *      *
AAG GAT TCC AGT TTT GAA CAA CAC AGT GTC CAA ATA ATG GTC AAG GAT
TTC CTA AGG TCA AAA CTT GTT GTG TCA CAG GTT TAT TAC CAG TTC CTA
Lys Asp Ser Ser Phe Glu Gln His Ser Val Gln Ile Met Val Lys Asp>

      630      640      650      660      670
      *      *      *      *      *      *      *      *
AAT GCA GGA AAA ATT AAA CCA TCC TTC AAT ATA GTG CCT TTA ACT TCC
TTA CGT CCT TTT TAA TTT GGT AGG AAG TTA TAT CAC GGA AAT TGA AGG
Asn Ala Gly Lys Ile Lys Pro Ser Phe Asn Ile Val Pro Leu Thr Ser>

      680      690      700      710      720
      *      *      *      *      *      *      *      *
CGT GTG AAA CCT GAT CCT CCA CAT ATT AAA AAC CTC TCC TTC CAC AAT
GCA CAC TTT GGA CTA GGA GGT GTA TAA TTT TTG GAG AGG AAG GTG TTA
Arg Val Lys Pro Asp Pro Pro His Ile Lys Asn Leu Ser Phe His Asn>

      730      740      750      760
      *      *      *      *      *      *      *      *
GAT GAC CTA TAT GTG CAA TGG GAG AAT CCA CAG AAT TTT ATT AGC AGA
CTA CTG GAT ATA CAC GTT ACC CTC TTA GGT GTC TTA AAA TAA TCG TCT
Asp Asp Leu Tyr Val Gln Trp Glu Asn Pro Gln Asn Phe Ile Ser Arg>

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Fig.32C.

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770          780          790          800          810
*           *           *           *           *
TGC CTA TTT TAT GAA GTA GAA GTC AAT AAC AGC CAA ACT GAG ACA CAT
ACG GAT AAA ATA CTT CAT CTT CAG TTA TTG TCG GTT TGA CTC TGT GTA
Cys Leu Phe Tyr Glu Val Glu Val Asn Asn Ser Gln Thr Glu Thr His>

      820          830          840          850          860
      *           *           *           *           *
AAT GTT TTC TAC GTC CAA GAG GCT AAA TGT GAG AAT CCA GAA TTT GAG
TTA CAA AAG ATG CAG GTT CTC CGA TTT ACA CTC TTA GGT CTT AAA CTC
Asn Val Phe Tyr Val Gln Glu Ala Lys Cys Glu Asn Pro Glu Phe Glu>

      870          880          890          900          910
*           *           *           *           *
AGA AAT GTG GAG AAT ACA TCT TGT TTC ATG GTC CCT GGT GTT CTT CCT
TCT TTA CAC CTC TTA TGT AGA ACA AAG TAC CAG GGA CCA CAA GAA GGA
Arg Asn Val Glu Asn Thr Ser Cys Phe Met Val Pro Gly Val Leu Pro>

      920          930          940          950          960
*           *           *           *           *
GAT ACT TTG AAC ACA GTC AGA ATA AGA GTC AAA ACA AAT AAG TTA TGC
CTA TGA AAC TTG TGT CAG TCT TAT TCT CAG TTT TGT TTA TTC AAT ACG
Asp Thr Leu Asn Thr Val Arg Ile Arg Val Lys Thr Asn Lys Leu Cys>

      970          980          990          1000
*           *           *           *           *
TAT GAG GAT GAC AAA CTC TGG AGT AAT TGG AGC CAA GAA ATG AGT ATA
ATA CTC CTA CTG TTT GAG ACC TCA TTA ACC TCG GTT CTT TAC TCA TAT
Tyr Glu Asp Asp Lys Leu Trp Ser Asn Trp Ser Gln Glu Met Ser Ile>

1010          1020          1030          1040          1050
*           *           *           *           *
GGT AAG AAG CGC AAT TCC ACA GGC GCG CCT AGT GGT GGA GGT GGC CGG
CCA TTC TTC GCG TTA AGG TGT CCG CGC GGA TCA CCA CCT CCA CCG GCC
Gly Lys Lys Arg Asn Ser Thr Gly Ala Pro Ser Gly Gly Gly Gly Arg>

      1060          1070          1080          1090          1100
*           *           *           *           *
CCC GCA AGC TCT GGG AAC ATG AAG GTC TTG CAG GAG CCC ACC TGC GTC
GGG CGT TCG AGA CCC TTG TAC TTC CAG AAC GTC CTC GGG TGG ACG CAG
Pro Ala Ser Ser Gly Asn Met Lys Val Leu Gln Glu Pro Thr Cys Val>

      1110          1120          1130          1140          1150
*           *           *           *           *
TCC GAC TAC ATG AGC ATC TCT ACT TGC GAG TGG AAG ATG AAT GGT CCC
AGG CTG ATG TAC TCG TAG AGA TGA ACG CTC ACC TTC TAC TTA CCA GGG
Ser Asp Tyr Met Ser Ile Ser Thr Cys Glu Trp Lys Met Asn Gly Pro>

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Fig.32D.

1160				1170				1180				1190				1200			
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		
ACC	AAT	TGC	AGC	ACC	GAG	CTC	CGC	CTG	TTG	TAC	CAG	CTG	GTT	TTT	CTG				
TGG	TTA	ACG	TCG	TGG	CTC	GAG	GCG	GAC	AAC	ATG	GTC	GAC	CAA	AAA	GAC				
Thr	Asn	Cys	Ser	Thr	Glu	Leu	Arg	Leu	Leu	Tyr	Gln	Leu	Val	Phe	Leu>				

1210				1220				1230				1240			
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
CTC	TCC	GAA	GCC	CAC	ACG	TGT	ATC	CCT	GAG	AAC	AAC	GGA	GGC	GGG	
GAG	AGG	CTT	CGG	GTG	TGC	ACA	TAG	GGA	CTC	TTG	TTG	CCT	CCG	CGC	
Leu	Ser	Glu	Ala	His	Thr	Cys	Ile	Pro	Glu	Asn	Asn	Gly	Gly	Ala	

1250				1260				1270				1280				1290			
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		
TGC	GTG	TGC	CAC	CTG	CTC	ATG	GAT	GAC	GTG	GTC	AGT	GCG	GAT	AAC	TAT				
ACG	CAC	ACG	GTG	GAC	GAG	TAC	CTA	CTG	CAC	CAG	TCA	CGC	CTA	TTG	ATA				
Cys	Val	Cys	His	Leu	Leu	Met	Asp	Asp	Val	Val	Ser	Ala	Asp	Asn	Tyr>				

1300				1310				1320				1330				1340			
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		
ACA	CTG	GAC	CTG	TGG	GCT	GGG	CAG	CAG	CTG	CTG	TGG	AAG	GGC	TCC	TTC				
TGT	GAC	CTG	GAC	ACC	CGA	CCC	GTC	GTC	GAC	GAC	ACC	TTC	CCG	AGG	AAG				
Thr	Leu	Asp	Leu	Trp	Ala	Gly	Gln	Gln	Leu	Leu	Trp	Lys	Gly	Ser	Phe>				

1350				1360				1370				1380				1390			
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		
AAG	CCC	AGC	GAG	CAT	GTG	AAA	CCC	AGG	GCC	CCA	GGA	AAC	CTG	ACA	GTT				
TTC	GGG	TCG	CTC	GTA	CAC	TTT	GGG	TCC	CGG	GGT	CCT	TTG	GAC	TGT	CAA				
Lys	Pro	Ser	Glu	His	Val	Lys	Pro	Arg	Ala	Pro	Gly	Asn	Leu	Thr	Val>				

1400				1410				1420				1430				1440			
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		
CAC	ACC	AAT	GTC	TCC	GAC	ACT	CTG	CTG	CTG	ACC	TGG	AGC	AAC	CCG	TAT				
GTG	TGG	TTA	CAG	AGG	CTG	TGA	GAC	GAC	GAC	TGG	ACC	TCG	TTG	GGC	ATA				
His	Thr	Asn	Val	Ser	Asp	Thr	Leu	Leu	Leu	Thr	Trp	Ser	Asn	Pro	Tyr>				

1450				1460				1470				1480			
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
CCC	CCT	GAC	AAT	TAC	CTG	TAT	AAT	CAT	CTC	ACC	TAT	GCA	GTC	AAC	
GGG	GGA	CTG	TTA	ATG	GAC	ATA	TTA	GTA	GAG	TGG	ATA	CGT	CAG	TTG	
Pro	Pro	Asp	Asn	Tyr	Leu	Tyr	Asn	His	Leu	Thr	Tyr	Ala	Val	Asn	

1490				1500				1510				1520				1530			
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*			
TGG	AGT	GAA	AAC	GAC	CCG	GCA	GAT	TTC	AGA	ATC	TAT	AAC	GTG	ACC	TAC				
ACC	TCA	CTT	TTG	CTG	GGC	CGT	CTA	AAG	TCT	TAG	ATA	TTG	CAC	TGG	ATG				
Trp	Ser	Glu	Asn	Asp	Pro	Ala	Asp	Phe	Arg	Ile	Tyr	Asn	Val	Thr	Tyr>				

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Fig.32E.

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1540      1550      1560      1570      1580
  *      *      *      *      *
CTA GAA CCC TCC CTC CGC ATC GCA GCC AGC ACC CTG AAG TCT GGG ATT
GAT CTT GGG AGG GAG GCG TAG CGT CGG TCG TGG GAC TTC AGA CCC TAA
Leu Glu Pro Ser Leu Arg Ile Ala Ala Ser Thr Leu Lys Ser Gly Ile>

      1590      1600      1610      1620      1630
  *      *      *      *      *
TCC TAC AGG GCA CGG GTG AGG GCC TGG GCT CAG TGC TAT AAC ACC ACC
AGG ATG TCC CGT GCC CAC TCC CGG ACC CGA GTC ACG ATA TTG TGG TGG
Ser Tyr Arg Ala Arg Val Arg Ala Trp Ala Gln Cys Tyr Asn Thr Thr>

      1640      1650      1660      1670      1680
  *      *      *      *      *
TGG AGT GAG TGG AGC CCC AGC ACC AAG TGG CAC AAC TCC TAC AGG GAG
ACC TCA CTC ACC TCG GGG TCG TGG TTC ACC GTG TTG AGG ATG TCC CTC
Trp Ser Glu Trp Ser Pro Ser Thr Lys Trp His Asn Ser Tyr Arg Glu>

      1690      1700      1710      1720
  *      *      *      *
CCC TTC GAG CAG TCC GGA GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA
GGG AAG CTC GTC AGG CCT CTG TTT TGA GTG TGT ACG GGT GGC ACG GGT
Pro Phe Glu Gln Ser Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro>

1730      1740      1750      1760      1770
  *      *      *      *      *
GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA
CGT GGA CTT GAG GAC CCC CCT GGC AGT CAG AAG GAG AAG GGG GGT TTT
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys>

      1780      1790      1800      1810      1820
  *      *      *      *      *
CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG
GGG TTC CTG TGG GAG TAC TAG AGG GCC TGG GGA CTC CAG TGT ACG CAC
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val>

      1830      1840      1850      1860      1870
  *      *      *      *      *
GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC
CAC CAC CTG CAC TCG GTG CTT CTG GGA CTC CAG TTC AAG TTG ACC ATG
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr>

      1880      1890      1900      1910      1920
  *      *      *      *      *
GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG
CAC CTG CCG CAC CTC CAC GTA TTA CGG TTC TGT TTC GGC GCC CTC CTC
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu>

```

Fig.32F.

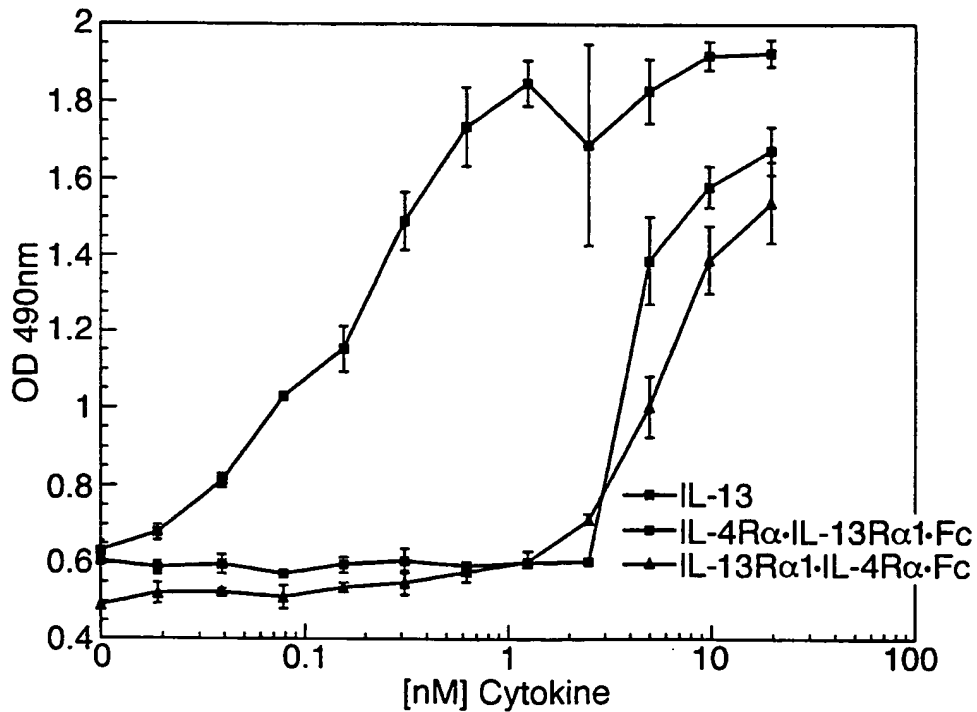
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Fig.32G.

2310			2320			2330			2340			2350		
*	*	*	*	*	*	*	*	*	*	*	*	*	*	
TTC	TCA	TGC	TCC	GTG	ATG	CAT	GAG	GCT	CTG	CAC	AAC	CAC	TAC	
AAG	AGT	ACG	AGG	CAC	TAC	GTA	CTC	CGA	GAC	GTG	TTG	GTG	ATG	
Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	
			2360			2370			2380					
			*	*	*	*	*	*						
			AAG	AGC	CTC	TCC	CTG	TCT	CCG	GGT	AAA	TGA		
			TTC	TCG	GAG	AGG	GAC	AGA	GGC	CCA	TTT	ACT		
			Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	***>		

Fig.33.



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Fig.34.

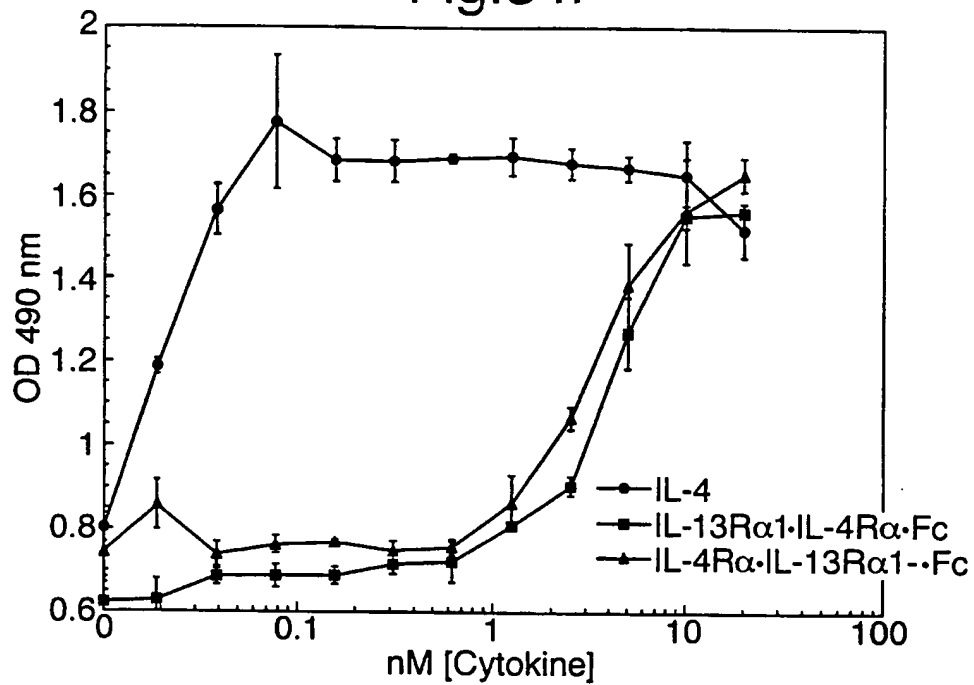
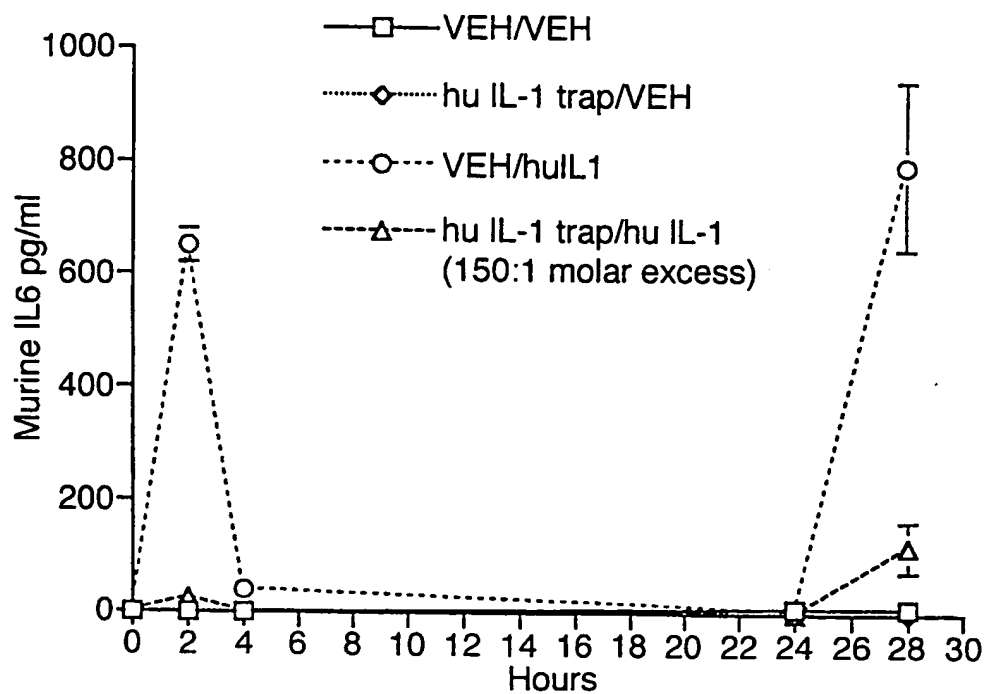


Fig.35.



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Fig.36A.

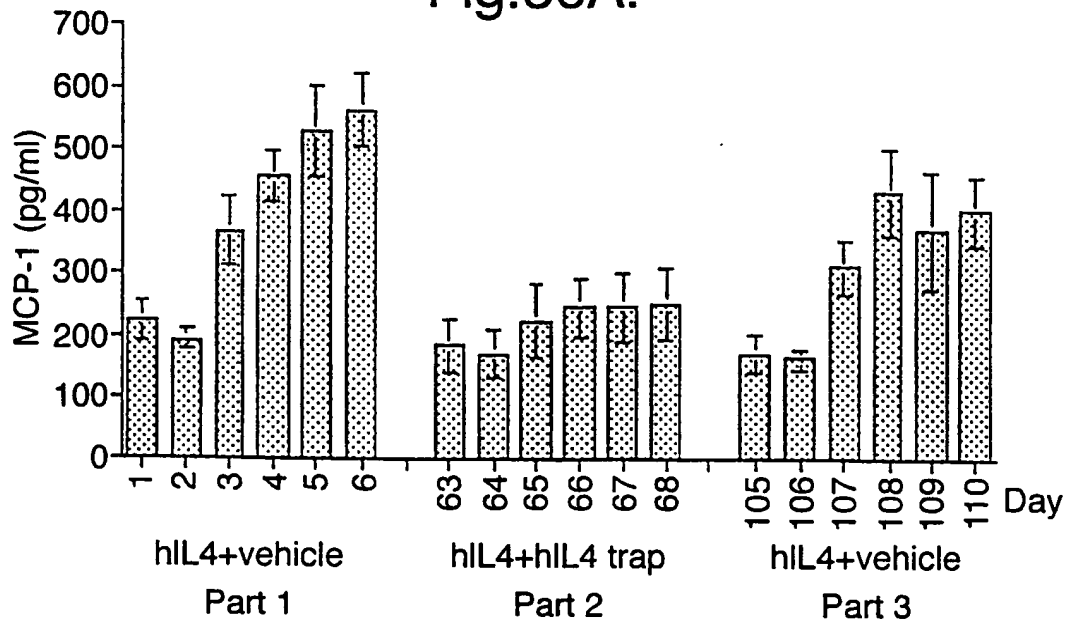
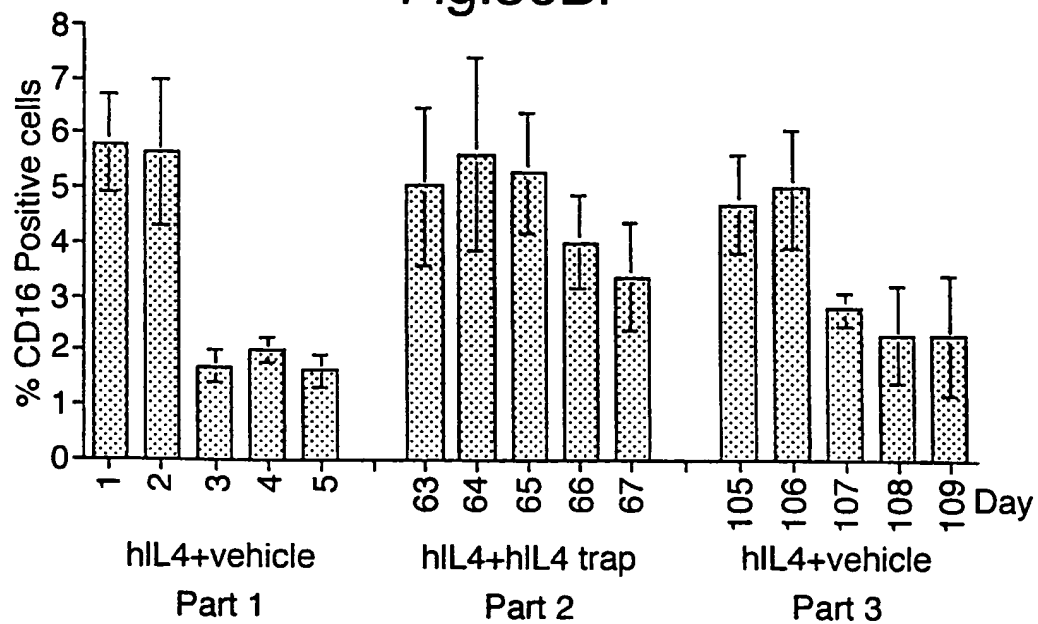


Fig.36B.



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Fig.37.

